ANNUAL REPORT

Division of Intramural Research Programs
National Institute of Mental Health

October 1, 1986 - September 30, 1987

VOLUME II PART 2
INDIVIDUAL PROJECT REPORTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs

ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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LABORATORY OF PRECLINICAL PHARMACOLOGY

CLINICAL BRAIN DISORDERS BRANCH

DIVISION OF INTRAMURAL RESEARCH PROGRAM

NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02244-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Effects of Neurotoxic Substances and Their Neurochemical Correlates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH; Dr. Richard Rothman, Guest Worker, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. De-Maw Chuang, Laboratory of Preclinical Pharmacology, IRP, NIMH; Dr. Michael Iadarola, Staff Fellow, National Institute of Dental Research; Dr. Anthony Adinolfi, University of California, Los Angeles, California

COOPERATING UNITS *(if any)*

National Institute of Dental Research (NIDR); University of California, Los Angeles, California

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated because the Principal Investigator left NIMH.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02245-03 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Consequences of Experimental Nerve Lesions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
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Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Aging

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.33	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Crush lesions were made on sciatic nerves of rats. We examined the influence on recovery of the time elapsed between successive injuries and of location and number of damaged sites. The results were assessed over a post-operative period of 2.5 months by studying tracks obtained from walking rats. This study demonstrated the central role of basal lamina tube lesions in nerve injuries. In contrast, neither injury to the tissue of the nerve (Schwann cells), nor to the neurite itself influenced the functional outcome.

Proposed Course of Project:

This project has been terminated because the Principal Investigator left NIMH. No new progress was made except for publications as listed.

Publications:

de Medinaceli, L., Quach, T., Duchemin, A.M. and Wyatt, R.J.: Is vigor of regeneration a key factor in the functional recovery of peripheral nerve injuries? Exp. Neurol. 94:788-790, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02246-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Post-Traumatic Autoimmune Reaction In Peripheral Nerve

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Luis de Medinaceli, M.D., Visiting Scientist, Neuropsychiatry Branch, IRP, NIMH

Dr. Yen-Nung Wang, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS.	PROFESSIONAL:	OTHER:
1.0	0.33	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study was conducted to show whether local autoimmune reactions can be observed after injury to the sciatic nerve in the rat. Furthermore, we attempted to correlate the intensity of the immunological reaction with the severity of nerve damage, the type of surgical treatment and the degree of functional recovery. A direct influence of autoimmunization was found. Its effect on functional recovery was moderate but constant.

PHS 6040 (Rev 1/84)

GPO 914-918

Proposed Course of Project:

This project has been terminated because the Principal Investigator left NIMH. No new progress was made.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02247-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prediction of Outcome of Peripheral Nerve Injuries - A Probability Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Luis de Medinaceli, M.D., Visiting Scientist, Neuropsychiatry Branch, IRP, NIMH

Dr. Robert R. Rawlings, Mathematical Statistician, Division of Biometry and Epidemiology, NIAAA; Dr. Yen-Nung Wang, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

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Division of Biometry and Epidemiology, NIAAA

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INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.33	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The long term functional consequences of peripheral nerve injuries are notoriously unpredictable. We hypothesized that considering the individual regrowth of the elementary components of a nerve (the neurites) rather than the global regeneration of the organ could lead to a better understanding of the mechanisms of nerve repair.

We postulated that the regrowth of any individual neurite can be defined in terms of its influence on recovery, the three main possibilities being valid, neutral and invalid regrowth. We have designed a probability model describing the prospects of regrowth for nerve composed of several types of fibers. This model is being tested in pre-determined situations to judge its validity. We found that possible variations in the outcome of nerve injuries could be explained by a parsimonious hypothesis: the randomness of regrowth.

PHS 6040 (Rev. 1/84) GPO 914-918

Proposed Course of Project:

This project has been terminated because the Principal Investigator left NIMH. No new progress was made except for publications as listed.

Publications:

de Medinaceli, L. and Rawlings, R.R.: Is it possible to predict the outcome of peripheral nerve injuries? A probability model based on prospects for regenerating neurites. BioSystems, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02250-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification of Messenger RNAs Encoding for Neurotrophic Factors in the Rat Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Anne-Marie Duchemin, M.D., Visiting Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Bruce K. Schrier, Laboratory of Developmental Neurobiology, NICHD, NIH

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Neuropsychiatry Branch

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NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotrophic factors have been shown to appear in the rat brain after lesion. This project intends the molecular cloning of the gene encoding for these lesion-induced trophic factors in the brain. First, we developed the assays to produce these factors and test for their neurotrophic activity. A fraction of mRNA, prepared from lesioned rat brain, responsible for neurotrophic activity was found by in vivo translation into proteins in Xenopus Laevis oocytes. This fraction is now being used to construct a cDNA library in the vector pGEM blue. We also contructed a cDNA library specific for lesioned rat brain in the vector bluescribe M 13. By differential colony hybridization, we have selected positive clones and some of these have been tested for coding for neurotrophic activity.

Project Description:

Objectives: Nerve growth factor (NGF) is the only neurotrophic factor with an established physiological role. During the last several years, the existence of other neurotrophic factors has been demonstrated. It has also been suggested that following injury of the central nervous system, neurotrophic and neurite promoting factors are made available to facilitate repair.

The purpose of this project was the purification of one of these neurotrophic factors which seem to be produced in the brain after injury. These factors could be useful for increasing the functional effects of brain tissue transplantations. One of the major obstacles to clinical applications of brain grafts is their limited efficacy. The availability of neurotrophic factors could enhance growth of the grafts.

Methods Employed:

(A) Techniques for assaying neurotrophic activity:

Neurotrophic activity is assayed on cell cultures of sympathetic neurons from 12-day-old chicken embryos.

(B) Source of neurotrophic factor:

We used injured brains of rats as a source of neurotrophic factors. The periphery of a vacuum aspiration wound of the cortex was removed seven days after lesion and was used as a source of neurotrophic factors and of corresponding messenger RNA.

(C) In vivo translation in Xenopus Laevis oocytes:

Xenopus laevis occytes have been used for an in vivo translation of mRNA into proteins. After fractionation of mRNA from lesioned rat brain on a sucrose gradient, mRNA fractions were injected into oocytes and the translation product tested for neurotrophic activity.

(D) Molecular cloning:

Different techniques have been used: cDNA synthesis, hybridization-subtraction, cloning in Bluescribe M13 and PGEM blue vectors, colony hybridization, and transcription.

Major Findings: In vivo translation into oocytes has allowed us to select a fraction of mRNA which seemed to encode for a neurotrophic factor. This mRNA is of high molecular weight and seems to be at a very low level in control animals. We are using mRNA from lesioned rat brain, selected by the in vivo translation, to make a cDNA library. This library is constructed with the Okayama and Berg method (to get full length cDNA inserts) in the expression vector pGEM blue.

Also, cDNA has been synthesized using mRNA from lesioned rat brain as a template. This cDNA has been hybridized with mRNA from control rats to remove common sequences. After this subtraction-hybridization, the sequences specific for the lesioned brain were inserted into the vector Bluescribe M13 and used to transform E. Coli. The cDNA library obtained contained about 800 positive clones. The specific clones were selected by differential colony hybridization with ^{32}P-cDNA from control and lesioned rat brains. The lesion-specific clones have been used to synthesize high activity mRNA transcripts. Several positive clones were grown individually and the synthesis of the fusion-protein induced by IPTG. The bacteria were then collected and homogenized and homogenates tested for

neurotrophic activity on cultures of 12 day old chicken embryo sympathetic neurons. Protein extract from one clone has been found able to support neuron survival and has been grown in larger quantity to prepare purified plasmid DNA. Transcripts from this plasmid will be used to probe Northern blots of mRNA extracted from control and from lesioned brain in order to confirm its specificity and further analyze its characteristics. It is now being sequenced.

Significance to Mental Health Research: We look forward to obtaining data from this project that may be applied to the understanding and treatment of the degenerative diseases of the CNS and recovery of patients with brain injury.

Proposed Course of Project: After characterization of the cDNA libraries, lesion-specific clones will be studied further. The assay for neurotrophic activity will be used to select the clones which could encode for neurotrophic factors. Then, the structure of the proteins and sequence of the corresponding genes will be analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02251-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Distribution of Brain Somatostatin mRNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Anita Feenstra, Ph.D., Visiting Associate, NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

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NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initiated a study of the local distribution of somatostatin mRNA in brains of patients with schizophrenia and Huntington's disease. The mRNA extraction method was adopted for brain tissue. Brains of patients and normal brains are being collected, and the proper area's are dissected.

PHS 6040 (Rev. 1/84)

GPO 914-918

Proposed Course of Project:

This project has been terminated. No new progress was made.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02252-03 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Behavioral Pharmacology and Toxicology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Renaud de Beaurepaire, Visiting Associate, NPB, IRP, NIMH; Dr. Jack A. Grebb, Laboratory of Molecular and Cellular Neuroscience, Rockfeller University, New York; Dr. Richard Shelton, Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, Tenn.; Dr. Saul Schwarcz, Department of Neurosurgery, Naval Medical Center, Bethesda, Maryland

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Neuropsychiatry Branch

SECTION
Preclinical Neurosciences Section

INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	0.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project on behavioral pharmacology and toxicology is aimed at the elucidation of neuropharmacological processes involved in abnormal brain function and at the development of techniques to intervene in abnormal brain function through pharmacological manipulations. These studies involve the induction of abnormal behavioral and physiological states through administration of drugs and other maniputations, and attempts to alter behavior and physiological states by pharmacological manipulations. Particular topics of interest are areas known to be associated with the actions of neuroleptic drugs and drug-induced psychoses. These include (i) phencyclidine, which in humans can induce abnormalities resembling schizophrenia; (ii) calcitonin and calcium-channel inhibitors and activators, are under investigation because of the possible relationships between calcium mechanisms and long-term changes in neuronal function induced by chronic neuroleptics; (iii) seizures and other phenomena related to the quisqualate type of glutamate receptors are of particular interest because of the close association between glutamate synapses and dopamine synapses on striatal neurons; (iv) changes in various neuronal systems particularly excitatory amino acid systems induced by chronic neuroleptic administration are investigated in order to contribute to an understanding of the mechanisms of action of neuroleptics.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: This research program is aimed at the development of animal models of brain dysfunction and development arrest induced by pharmacological agents, as well as the development of pharmacological agents to alleviate the behavioral manifestations of these manipulations.

Methods Employed: Behavioral studies include observations of seizures, measurements of general locomotor activity, tests of maze-learning and operant conditioning, measurements of rotational behavior and measurements of feeding and drinking behavior. Animals will also be subject to the induction of brain lesions through the administration of drugs and neurotoxins as well as stereotaxic injection of neurotoxic substances. Histological studies are also performed for some experiments.

A. Behavioral Pharmacology of Phencyclidine

Objectives: This research program is aimed at the development of animal models for the measurement of behavioral responses to phencyclidine (PCP), employment of these models to assess the ability of various agents to antagonize the behavioral effects of PCP, and to characterize the behavioral pharmacology of PCP. The immediate purpose of the experiments is the development of pharmacological agents for the treatment of adverse reactions of PCP abuse. The long-range goal is the development of agents for the treatment of schizophrenia and/or elucidation of causes of schizophrenia.

Methods Employed: Behavioral observations and testing for general locomotor activity following systemic or intracerebral administration of PCP.

Major Past Findings: Phencyclidine, or PCP, is considered to be one of the best pharmacological models of schizophrenia because of the wide range of psychological reactions to PCP abuse, including sensory disturbances (rather than vivid visual hallucinations as with LSD) as well as activation similar to that produced by amphetamine. In addition, violent and even prolonged psychotic reactions to PCP abuse are common. In initial studies a large series of pharmacological agents were screened for their ability to block PCP reactions in mice. Only a few agents, including phenothiazine neuroleptics, GABA agonists, and yohimbine and methysergide, were effective behavioral antagonists of PCP. Other agents were ineffective, including some that are used clinically such as diazepam and haloperidol. In a subsequent study, a variety of neuroleptics were tested for their ability to antagonize PCP. Significant differences among neuroleptics were found, with the most effective agents being methiothepin and fluphenazine, as well as phenothiazines in general, while haloperidol, pimozide, molindone, and sulpiride were relatively ineffective.

A study of the genetics of PCP reactions in recombinant inbred strains of mice has also been performed, for the purpose of developing animal models of severe vs. mild PCP reactions in man, as well as to determine whether reactivity to PCP is determined, and unrelated to responsivity to amphetamine. BALB strain mice showed pronounced reactions to PCP, while C57 Bl/6 mice reacted much less markedly (about one-third as much locomotor stimulation). Most of the recombinant strains showed intermediate reactions. The BALB strain mice may therefore provide a good model for severe PCP reaction in man. Both BALB and C57 Bl/6

strain mice were found to be similarly susceptible to blockade of PCP-induced stimulation by haloperidol.

New Findings: No new studies this year.

Proposed Course of Subproject A: Studies of new neurotransmitter antagonists or agents with suspected neuroleptic activity may be tested as antagonists of PCP-induced stimulation as well as amphetamine-induced stimulation if warranted by other studies in the Neuropsychiatry Branch.

B. Seizures and Amino Acids

The excitatory amino acids, glutamate and aspartate, and their decarboxylated inhibitory counterparts GABA and glycine, are major and ubiquitous putative regulators of neuronal excitation and inhibition. Excitatory neurotransmitters potentially may be involved in neuropsychiatric disorders in two distinct ways: (1) Disturbances in amino acid neurotransmitter function may be involved directly in epilepsy, and possibly in schizophrenia. Schizophrenia is exacerbated by administration of amino acids such as methionine, and there is some evidence that the effects of methionine are due to metabolic conversion to the excitatory substance homocysteine. The disease homocystinuria is also accompanied by behavioral disturbances. (2) There is increasing evidence that injury to neurons which is caused by excess stimulation by excitatory amino acids, such as glutamate or kainic acid, is a major mechanism of CNS neurotoxicity, and has been hypothesized to be involved in Huntington's chorea as well as the neuronal damage consequent to ischemia. Thus, there is a potential involvement of excitatory amino acid toxicity in schizophrenia and other neuropsychiatric disorders as well.

Methods Employed: These studies are conducted primarily by administration of drugs, either systemically or intracerebrally into the lateral ventricles, followed by observation and blind scoring of seizures. Seizures are induced by chemical agents, auditory stimulation, or electrical stimulation of the brain. Current studies also involve induction of seizures by administration of amino acid agonists, such as quisqualate, directly into the cerebral ventricles. Some experiments involve administration of excitatory substances to mice on neonatal day I followed by measurement of weight gain and histological assessment of neuronal damage.

Major Past Findings: A considerable body of literature in the past has shown that schizophrenia can be exacerbated by administration of the amino acid methionine. We obtained evidence from animal studies that some of the effects of methionine appeared to be due to accumulation of its metabolite homocysteine, rather than to increases in methylation reactions as was originally supposed. Evidence has also been obtained that homocysteine is a relatively specific agonist of the quisqualate-sensitive or "Type II" excitatory amino acid receptor site. Glutamic acid diethyl ester (GDEE), a quisqualate antagonist, also antagonized homocysteine-induced seizures. Betaine, which is involved in the remethylation of homocysteine, has also been found to have anticonvulsant properties. This anticonvulsant effect is mediated by the central nervous system, and appears to be a pharmacological effect, rather than a metabolic effect, because its metabolically-inactive metabolites sarcosine and dimethylglycine have effects similar to those of betaine. Homocystinuria, a disorder involving excess accumulation of homocysteine and related amino acids in addition

to mental retardation and seizures, has been reported by others to be successfully treated with betaine. This area is also of interest as induction of brain damage by excessive excitatory amino acids has recently been recognized as a major neurotoxicological process and could be involved in the genesis of developmentally-related brain abnormalities.

New Findings:

1. A model for the induction of seizures by the direct intracerebral administration of quisqualic acid was developed. These seizures were found to be blocked by systemic GDEE. Quisqualate-induced seizures were blocked by valproic acid, but most other anticonvulsant drugs had no effect. Diazepam partially antagonized quisqualate-induced seizures in high dosages. These data are consistent with the hypothesis that the quisqualate-sensitive receptor is involved in some forms of seizure phenomena, particularly those which can specifically be blocked by valproic acid.
2. Structure-activity studies of GDEE suggest that deaminated derivatives of GDEE possess anticonvulsant activity equal to or greater than that of GDEE. Increasing carbon chain lengths by more than one over that of GDEE resulted in decreased activity. Methyl esters were less potent than ethyl esters.
3. GDEE was examined for induction of ataxia as a general measure of toxicity. Whereas most other anticonvulsants as well as other types of excitatory amino acid antagonists induced severe ataxia in dosages about two-fold larger than the anticonvulsant dosages, GDEE did not produce ataxia at any dosage tested. Although GDEE is not very potent on a mg/kg basis, these data suggest that more potent derivatives of GDEE might have useful anticonvulsant activity.
4. A model for the induction of seizures by the direct activation of central calcium channels with the calcium-channel agonist BAY K-8644 has been developed. The activation of central calcium channels can induce seizures which can be altered by chronic treatment with calcium channel blockers. More recently, the methods for evaluation of BAY K-8644-induced seizures have been revised in order to separate several different components of the seizure phenomena. BAY K-8644-induced seizures have been found to be extremely resistent to anticonvulsants, but easily blocked by calcium channel antagonists. Drugs which alter calcium metabolism, such as chlorpromazine, alter but do not block BAY K-8644-induced seizures. This model of calcium-related epileptogenesis may help to elucidate the relative role of calcium in the actions of various convulsant and anticonvulsant drugs.

Proposed Course of Subproject B: The concept of induction of seizures by the direct intracerebral application of specific agents with known actions may result in the development of a set of seizure models, each with known specific properties. It may therefore become possible to examine pharmacological agents with potential antiseizure activity against this set of models and to develop a more accurate preclinical pharmacological characterization than is generally obtained by global models such as pentylenetetrazol and electroconvulsive shock-induced seizures. Moreover, the methods and information obtained in these studies are further applied in studies of chronic neuroleptic effects described under Subproject E: Chronic Neuroleptic Studies.

C. Developmental Arrest

Developmental arrest consists of brief interference with development of the central nervous system, usually induced by the administration of short-acting antimitotic agents during critical periods of brain development. Such models can

be employed to produce diffuse but relatively restricted brain abnormalities, such as reductions in cortical thickness or volume of the striatum. Administration of the antimitotic agent methylazoxymethanol (MAM) to rats on the fifteenth day of gestation, for example, interferes with cortical development and induces behavioral hyperactivity, putative deficits in learning, and hyperinnervation of the cerebral cortex by catecholaminergic fibers. The potential relevance of this model to neuropsychiatric disorders has prompted us to conduct additional studies.

Major Past Findings: Developmental arrest induced by the antimitotic agent MAM serves as a model of abnormal development due to brief interference with CNS growth. When administered during growth of the cerebral cortex, the cerebral cortex does not fully develop, leading to a variety of behavioral abnormalities. This model may parallel some of the minor abnormalities of CNS structure recently reported in schizophrenia. One of the concomitants of this model is an excessively dense catecholaminergic innervation of the cerebral cortex.

We have previously measured a variety of behavioral indices in animals with developmental arrest induced by MAM. Essentially all of the observed abnormalities could be explained by the presence of hyperactivity in the animals which had been treated by MAM; for example, learning abnormalities were present only when the learning tasks required increased behavioral output for successful performance.

New Findings: Studies of maze-learning capacity of animals prenatally treated with MAM are completed. It is hoped that maze learning will provide a measure of learning that is relatively independent of levels of activity.

Measurements of response of MAM-treated rats to amphetamine have also been conducted. Data analysis and histological studies for this project are still in progress.

Course of Subproject C: As information about CNS pathology and brain atrophy in schizophrenia continues to develop, attempts to produce a developmental arrest model of schizophrenia may receive additional impetus. For example, the MAM model may be applied to test for effects of chronic neuroleptics on cortico-striatal system (see Subproject E).

D. Calcitonin

Calcitonin, a peptide hormone secreted by the C-cells in the thyroid, is primarily involved in the regulation of peripheral calcium metabolism. Our group has found that calcitonin is also, however, a very potent inhibitor of eating behavior, and this effect is mediated directly by the brain. Calcitonin has subsequently been reported to inhibit amphetamine-induced stimulatory effects. Even though calcitonin is not produced by the brain it may be an important peripherally-derived hormonal regulator of behavioral processes through actions on the central nervous system.

Methods Employed: Animals receive chronic cannula implants into various brain nuclei or into the lateral ventricle using standardized procedures. Calcitonin or other substances are then administered through the cannulae in small amounts, and eating behavior, activity, and other behavioral responses are measured. In

some experiments, the animals also receive systemic injections of d-amphetamine or calcitonin.

Major Past Findings: We have previously found that calcitonin is an extremely potent inhibitor of eating behavior in animals, and this effect has been found to be mediated via the CNS. Others have reported that calcitonin is capable of inhibiting amphetamine-induced behavioral stimulation. There is some evidence that calcitonin is a hormone that serves to modulate calcium uptake by certain CNS neurons.

The specific site of action of calcitonin within the CNS has been localized by measurement of behavioral responses to calcitonin following local intracerebral injections through chronically implanted cannulae. Responses to calcitonin were obtained from hypothalamic nuclei, especially the paraventricular nucleus, the perifornical area, the supraoptic nucleus, ventromedial nucleus, and nucleus reuniens. Responses were also obtained from the vertical limit of the diagonal band and from the nucleus accumbens. The peripheral effects of calcitonin are known to diminish with age. We found that the CNS effects did not decrease with age, suggesting that the CNS effects and peripheral effects are independent.

New Findings:

1. The intracerebral localization of the antagonism of amphetamine-induced hyperactivity by calcitonin has been examined. Calcitonin was found to inhibit amphetamine-induced activity when administered directly into several hypothalamic areas, including the paraventricular nucleus, the perifornical area, the floor of the hypothalamus (anterior), and the lateral hypothalamus, as well as in the nucleus accumbens and zona incerta.
2. The finding that calcitonin inhibited eating when administered into the floor by the hypothalamus was further examined, with the purpose of determining whether areas involved in the regulation of behavior are located in this region. Calcitonin, neurotension, bombesin, and cholecystokinin were locally administered into the floor of the hypothalamus and the animals were examined for changes in eating behavior and activity. Cholecystokinin had no effect in any of the areas where it was infused. Each of the other peptides were found to have potent behavioral effect in some parts of the floor of the hypothalamus, which differed for each of the peptides. These data suggest that some parts of the floor of the hypothalamus have an important role in behavioral regulation.

Proposed Course of Subproject D: Studies are planned to further investigate effects of calcitonin and calcitonin analogues on stimulant drugs with differing mechanisms of action, including amphetamine, apomorphine, and PCP. Studies of effects of calcitonin on BAY K-8644 induced seizures will be included under Subproject B.

E. Chronic Neuroleptic Studies

One of the most salient properties of neuroleptics is the gradual onset of antipsychotic effects after several weeks of treatment. Because the antipsychotic effect is delayed, the mode of action of neuroleptics cannot be entirely understood solely on the basis of acute studies. For that reason, a series of experiments on changes in responsivity to various pharmacological agents and brain neurotransmitter receptors following chronic drug treatment have been initiated. These studies may also be relevant to mechanisms for induction of tardive dyskinesia.

Major Past Findings: Previous studies have shown alterations in responsivity to GABAergic and cholinergic agents following chronic neuroleptic administration. Recent anatomical investigations (by other groups) have shown an intimate association between the glutamate-mediated corticostriatal pathway and the dopamine-mediated nigrostriatal pathway. The possibility of interactive changes involving these two systems is therefore being investigated.

New Studies: Experiments to evaluate behavioral responsivity to glutamate agonists and antagonists after chronic neuroleptic treatment are underway. Initial results suggest that chronic neuroleptic (haloperidol) administration attenuates the behavioral effects of quisqualic acid, a glutamate agonist. The seizures induced by quisqualate were not altered. The effects of the glutamate antagonist glutamate diethyl ester were also attenuated by chronic haloperidol treatment. Additional experiments to evaluate striatal glutamate receptors following chronic neuroleptic administration are also being developed.

Proposed Course of Subproject E: The course of these experiments will be re-evaluated upon completion of the experiments now in progress.

Significance to Mental Health Research: This program attempts to investigate some possible forms of abnormal brain function in animal models. In the realm of neurotoxicology, two of the most promising models are the excitotoxic model, i.e., the induction of neuronal death through the actions of excitatory amino acids or their analogues, and the particular sensitivity of the dopamine systems of the brain to neurotoxins, such as manganese and the recently discovered agent MPTP. Attempts to develop methods for further characterizing these models of neurotoxicity are underway. Other promising models include the study of other systems which interact with dopaminergic systems in the brain, such as excitatory amino acid mechanisms and calcium regulatory systems. As information about the pathology of the brain in schizophrenia continues to develop, it is anticipated that the various models can be refined and further developed.

Proposed Course of Behavioral Pharmacology and Toxicology Project: This project is expected to continue indefinitely, as an interactive process with clinical sections of the branch.

Publications:

Schwarz, S.S., and Freed, W.J.: Inhibition of quisqualate-induced seizures by glutamic acid diethyl ester and anti-epileptic drugs. J. Neural Transm. 67: 191-203, 1986.

Grebb, J.A., Shelton, R.C., and Freed, W.J.: Diltiazem or verapamil prevents haloperidol-induced apomorphine supersensitivity in mice. J. Neural Transm. 68: 241-255, 1987.

Shelton, R.C., Grebb, J.A., and Freed, W.J.: Induction of seizures in mice by the calcium channel agonist BAY K 8644. Brain Res., in press.

de Beaurepaire, R., and Freed, W.J.: Regional localization of the antagonism of amphetamine-induced hyperactivity by intracerebral calcitonin injections. Pharmacol. Biochem. Behav., in press.

de Beaurepaire, R., and Freed, W.J.: Anatomical mapping of the rat hypothalamus for calcitonin-induced anorexia. Pharmacol. Biochem. Behav., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02253-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Tissue Transplantation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

William J. Freed, Ph.D., Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Urmi Patel-Vaidya, Staff Fellow, NPB, IRP, NIMH; Renaud de Beaurepaire, Visiting Associate, NPB, IRP, NIMH; Dr. Herbert Geller, Rutgers University, New Brunswick, New Jersey; Dr. Jeff Laskin, Rutgers University, New Brunswick, New Jersey; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Saul Schwarz, Department of Neurosurgery, Naval Hospital, Bethesda, Maryland; Dr. Ronald Hargraves, Department of Neurosurgery, Naval Hospital, Bethesda, Maryland

COOPERATING UNITS *(if any)*

Rutgers University, New Jersey; Naval Medical Center, Bethesda, Maryland; University of Minnesota; University of Michigan

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	0.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies are primarily aimed at transplantation of catecholamine-containing tissues, including adrenal medulla, tumor cells, and embryonic brain tissue into the brain. The purpose of these experiments is to elucidate the properties of these tissues after transplantation and the response of the host brain to the transplanted tissues. Specifically, these experiments employ non-primate animal models to (1) develop the techniques of brain tissue transplantation for clinical use in Parkinson's disease; (2) develop brain tissue transplantation techniques so that they eventually may be applicable to other disorders, such as schizophrenia or Alzheimer's disease if and when these disorders become well enough understood to permit such applications; and (3) elucidate factors that control the development of the brain and responses of the brain to injury or impairment, with particular emphasis on the nigrostriatal dopamine system. During the past reporting year, significant progress has been made in these areas.

PHS 6040 (Rev 1/84)

GPO 914-918

Collaborators:

Dr. Steven McLoon, Department of Anatomy, University of Minnesota
Dr. Jill Becker, Department of Psychology, University of Michigan
Dr. Terrance Robinson, Department of Psychology, University of Michigan

Project Description:

Objectives: The major objective of this program is to develop brain tissue transplantation as a technique for the repair of localized damage to the central nervous system. There are three more specific objectives: (1) to develop the techniques of brain tissue transplantation so that it may be applied clinically to Parkinson's disease; (2) to develop brain tissue transplantation techniques for eventual application to other disorders, such as schizophrenia or Alzheimer's disease if and when these disorders become well enough understood to permit such applications; and (3) to employ brain tissue transplantation as a technique to elucidate factors that control the development and plasticity of the brain, particularly the nigrostriatal dopamine system.

Methods Employed: These studies involve surgical, behavioral and histological-histochemical procedures in animal subjects.

Major Past Findings: Grafts of embryonic substantia nigra or young adult adrenal medulla have been shown to decrease rotational behavior consequent to unilateral lesions of the substantia nigra (SN). The SN grafts produce dopamine and reinnervate the host caudate-putamen, and decrease spiroperidol binding in the striatum concomitant with their behavioral effects. The adrenal medulla grafts also produce dopamine, but do not reinnervate the host brain, apparently exerting their behavioral effects simply through secretion of catecholamines followed by diffusion into the host brain tissue. Although these intracerebral grafts survive indefinitely across major histocompatibility typings, it has been found to be possible to induce rejection through peripheral sensitization of the host animals. It should be emphasized that the behavioral effects of both adrenal medulla grafts and SN grafts are relatively limited in magnitude: In general, the SN grafts appear to be limited in their efficacy because of a limited reinnervation of the host brain, while the effects of adrenal medulla grafts are limited because of limited survival of the grafted cells.

Intrastriatal adrenal medulla grafts have been found to survive indefinitely, albeit to a limited extent. These grafts produce some behavioral effects, particularly when obtained from young donors. Attempts to improve the performance of substantia nigra grafts have been initiated, and are continuing. Substances such as gangliosides, haloperidol, and estrogen were found to have no effect on substantia nigra grafts.

1. Trophic effects of cortical lesions and striatal lesions on substantia nigra grafts: These studies are intended to exploit the possibility of secretion of trophic substances by damaged brain tissue to enhance the penetration of dopamine-containing fibers from SN grafts into the host brain tissue. In an initial experiment, cortical lesions were found to increase the growth of fibers from grafts into the host brain, but only in the most dorsal part of the striatum close to the lesioned brain area. Reinnervation of other parts of the striatum was not changed by lesions. Results of a long-term study also support this conclusion. These differences were not due to anatomical distortion of the brain from the lesions or to other anatomical artifacts. The cortical lesions themselves were also found to reduce rotational behavior by substantia nigra grafts. Another experiment, involving kainic acid lesions of the striatum, confirmed the stimulatory effect of brain lesions on reinnervation of the striatum by substantia nigra grafts. In contrast to cortical lesions, kainic acid lesions of the striatum tended to slightly enhance the behavioral effect of

grafts. Studies of cell survival in substantia nigra grafts and effects of other types of lesions are continuing.

2. Combined substantia nigra and striatal grafts: A study of the effects of combined grafts of substantia nigra and embryonic striatum into the lateral ventricle has been performed. The substantia nigra grafts were found to completely innervate the embryonic striatal grafts in preference to the host brain; when a striatal graft was present in the lateral ventricle, little or no innervation of the host striatum occurred. This study suggests that the mature denervated striatum is a relatively inferior target, as compared to immature striatum. This study also suggests that the limited efficacy of substantia nigra grafts is due to properties of the target tissue, rather than a limited efficacy of the substantia nigra grafts themselves.

3. Substantia nigra grafts in neonatal hosts: In order to exploit the favorable properties of embryonic striatum as a target tissue for substantia nigra grafts, a paradigm was devised so that embryonic substantia nigra was transplanted into the lateral ventricle of normal newborn rats within one day after birth (control animals received sciatic nerve grafts). The animals were then allowed to grow to maturity, and received bilateral lesions of the substantia nigra. The presence of neonatally-implanted substantia nigra grafts protected the animals against the development of aphagia, adipsia, akinesia, and rigidity induced by the SN lesions. Differences between substantia nigra-grafted rats and controls were very substantial; for example, the rats with substantia nigra grafts were 3.7 times as active as the controls. Surviving grafts were consistently found to be well-incorporated into the host striatum. Therefore the effectiveness of substantia nigra grafts can be increased by transplantation into neonatal hosts.

4. Trophic effects on intraparenchymal adrenal medulla grafts: Efforts have been directed at assessing trophic effects and implantation techniques for intraparenchymal grafts of adrenal medulla. Studies performed thus far include evaluation of the effects of co-implantation of adrenal medulla with tissues containing corticosteroids (adrenal cortex), nerve growth factor (NGF) (mouse submaxillary gland) or other unidentified trophic substances (rat iris), effects of prior lesions of the implantation site, assessment of graft survival in inbred rat strains (to rule out the possibility of partial rejection of the grafts), and the possible influence of trophic substances such as NGF. Some of these data have been decoded, and so far there have been some suggestive data but these findings have not been conclusive. These studies are still in progress at the present time.

5. Development of a cat model: In order to have available another model for development of brain grafting for application to higher-order species, we have taken steps to develop brain tissue transplantation procedures for cats. We have developed a number of behavioral testing procedures and have developed procedures for producing complete unilateral lesions of the substantia nigra. Two cats have so far received intraventricular adrenal medulla grafts. No changes in behavior have as yet been detected. Testing is, however, still in progress.

6. Chronic intrastriatal catecholamine infusions: In order to determine the actual rate of catecholamine secretion that would be required for adrenal medulla grafts to produce positive behavioral effects in animals with substantia nigra lesions, we have begun studies of chronic dopamine infusions using osmotic minipumps. Chronic dopamine infusions produce transient behavioral activation and stereotypy in both normal animals and animals with unilateral substantia nigra lesions, but produce a longer lasting suppression of apomorphine-induced rotational behavior. Diffusion of the infused dopamine is localized to within 2-3 mm of the infusion site. Dose-response studies and additional characterization

of these infusions are underway. Other studies include effects of intraventricular infusions and alternate buffer systems.

7. Catecholamine release from brain grafts: In collaboration with Drs. Jill Becker and Terrance Robinson of the University of Michigan, studies to measure catecholamine release from brain grafts using intracerebral dialysis probes have been initiated. The dialysis probes have been developed, as well as a technique for implanting brain grafts through chronic cannulae. Data obtained so far suggest that catecholamines released from adrenal medulla grafts in the lateral ventricle are adsorbed into the striatum. Amounts of catecholamines which are "washed away" in the cerebrospinal fluid are very minimal.

8. Grafts in non-lesioned hosts: Previous experiments found that substantia nigra grafts induced a hyperresponsivity to amphetamine. Experiments aimed at determining whether substantia nigra grafts induce hyper-normal behavioral responses to amphetamine in normal (non-lesioned) hosts have been initiated. Recipients are mature normal rats. Data obtained thus far suggest that these grafts do not alter responsivity to amphetamine in normal, non-lesioned animals.

9. Effects of laminin and collagen on brain grafts: Studies of the effects of laminin, a basal lamina component which stimulates neurite growth, and collagen, a non-specific supporting substance, on growth of substantia nigra and adrenal medulla grafts in the ventricle have been undertaken. Results suggest that implantation of these grafts in these media does not alter their effects on rotational behavior.

10. Effects of adrenalectomy and adrenal cortex on intraventricular adrenal medulla grafts: Adrenal corticosteroids are known to influence the differentiation of adrenal chromaffin cells. Studies on the development of adrenal medulla grafts under conditions of varying steroid hormone concentrations have therefore been initiated. No results are as yet available.

Significance to Mental Health Research: These studies may lead to the development of brain tissue transplantation as a therapeutic procedure for Parkinson's disease and eventually for other disorders. In addition, brain tissue transplantation is a valuable technique for the investigation of trophic functions in the brain. For example, the finding that brain injury has a trophic effect on dopamine-containing neurites is of potential importance for the understanding of the developmental and trophic influences on the brain dopaminergic system and its possible dysfunction in schizophrenia. Subsequent studies using this paradigm may provide useful information relating to the effects of brain injury on neuronal circuits. Investigation of trophic functions and their possible absence is of particular importance for diseases such as schizophrenia, which may involve relatively subtle forms of neuronal dysfunction rather than readily detectable brain atrophy or neuronal degeneration.

Proposed Course of Project: The investigation of brain tissue transplantation as a therapeutic technique is expected to continue until a reasonably effective procedure that can be applied to Parkinson's disease is developed. Subsequently and concurrently grafting will be studied primarily as a means of assessing trophic control of development and function of the brain dopaminergic systems. Studies of possible application of brain tissue transplantation to other disorders will also be continued in particular as developments in other fields elucidate possible applications.

Publications:

Freed, W.J., Patel-Vaidya, U., and Geller, H.M.: Properties of PC12 pheochromocytoma cells transplanted to the adult rat brain. Exp. Brain Res. 63: 557-566, 1986.

Freed, W.J., Cannon-Spoor, H.E., and Krauthamer, E.: Intrastriatal adrenal medulla grafts in rats: Long-term survival and behavioral effects. J. Neurosurgery 65: 664-670, 1986.

Wyatt, R.J., de Medinaceli, L., and Freed, W.J.: Functional repair of the nervous system: A focus on aging. In Bergener, M., Ermini, M. and Stahelin, H.B. (Eds.): The 1986 Sandoz Lectures in Gerentology: Dimensions of Aging. London, Academic Press, 1986, pp. 143-160.

Freed, W.J., and Wyatt, R.J.: Brain tissue transplantation in animal models of extrapyramidal motor dysfunction. Chapt. 48. In Meltzer, H. (Ed.): Psychopharmacology: A Third Generation of Progress. New York, Raven Press, 1987, pp. 471-479.

Schwarz, S.S., and Freed, W.J.: Brain tissue transplantation in neonatal rats prevents a lesion-induced syndrome of adipsia, aphagia, and akinesia. Exp. Brain Res. 65: 449-454, 1987.

de Beaurepaire, R., and Freed, W.J.: Embryonic substantia nigra grafts innervate embryonic striatal co-grafts in preference to mature host striatum. Exp. Neurol. 95: 448-454, 1987.

Hargraves, R.W., and Freed, W.J.: Chronic intrastriatal dopamine infusions in rats with unilateral lesions of the substantia nigra. Life Sci. 40: 959-966, 1987.

Freed, W.J., Cannon-Spoor, H.E., de Beaurepaire, R., Greenberg, J.A., and Schwarz, S.S.: Embryonic substantia nigra grafts: Factors controlling behavioral efficacy and reinnervation of the host striatum. In Azmitia, E. and Bjorklund, A. (Eds.): Cell and Tissue Transplantation into the Adult Brain, New York Academy of Sciences, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02255-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Calcium Channel Inhibitors: Interactions Systems - Human Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Llewellyn B. Bigelow, Associate Clinical Director for WAW Division, Saint Elizabeths Hospital; Dr. Edward Taylor, Clinical Social Worker, WAW Division, Saint Elizabeths Hospital, Dr. Richard Suddath, Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium channel inhibitors (CCI's) are thought to affect calcium flux through membrane bound channels as their major site of action. There are reports of clinical trials involving over 150 patients suggesting that CCI's also have beneficial effects in neuropsychiatric disorders. There are four major subclasses of CCI's; each appears to have unique combinations of biochemical and behavioral properties. Additional studies have suggested a complex interaction between dopamine receptor function and calcium channels. We have completed a study of the clinical effects of verapamil in a schizophrenic population where trends toward improvement did not reach statistical significance. We have proceeded with the protocol to examine the effects of nifedipine in a similar cohort. Results on the first eight completed subjects have shown two trends: 1) an increase in "negative" symptoms of schizophrenia while on active nifedipine, and 2) a decrease in abnormal involuntary movements on the AIMS (abnormal involuntary movement scale) while on active nifedipine. Also, a comparison of the placebo periods before and after active nifedipine suggests a significant sequence effect measured by continued worsening of the BPRS (brief psychiatric rating scale) scores after active nifedipine.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description:

Objectives: Examine the clinical effect of nifedipine in a schizophrenic population.

Methods Employed: The patients will have been diagnosed according to DSM-III, and will be followed with repeated neurological, neuropsychological, and psychiatric examinations as well as nursing BPRS.

Major Past Findings: Nifedipine-like drugs inhibited PCP-induced behavior in mice significantly better than did verapamil. Verapamil did not show statistically significant benefit in a cohort of schizophrenics.

New Findings: Nifedipine has shown a trend to cause an overall worsening of psychotic symptoms but an overall improvement in abnormal movements in patients fully evaluated to date.

Significance to Mental Health Research: There is a clear need for alternate medication for schizophreniform illnesses and the CCI's may provide this alternate with a side effect profile significantly different from the current standards.

Proposed Course of Project: The clinical trials with 8 subjects have been completed and at least 2 more subjects will be completed by August 1987. In addition, blood concentrations of haloperidol concurrent with the nifedipine protocol are being obtained in some patients. This may necessitate extension of the project up to June 1988 for testing and evaluation of a sufficient number of patients to adequately explore any significant drug interaction effects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02256-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Defect Symptoms in Schizophrenia: Their Measurement, Correlates, and Treatment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Darrell G. Kirch, M.D., Senior Staff Fellow, NPB, IRP, NIMH

Dr. Andrei C. Jaeger, New York State Psychiatric Institute; Dr. Edward H. Taylor, Clinical Social Worker, WAW Division, Saint Elizabeths Hospital; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

New York State Psychiatric Institute

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Renewed interest in the role of negative symptoms in "defect state" schizophrenia encouraged us to develop a "Negative Symptom Rating Scale (NSRS)" to more efficiently measure this syndrome. A study has been conducted performing a factor analysis of the NSRS. Studies are being conducted to explore the relationship between schizophrenia, social intelligence, general intelligence, negative symptoms and premorbid social functioning in schizophrenic patients.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: Patients with schizophrenia are known to have social withdrawal, impaired social judgement, difficulty with problem solving, and defective motivation. This has been referred to as the "defect state" and these symptoms have been labeled "negative symptoms." The goals of this project are to characterize negative symptoms in patients with chronic schizophrenia using a rating instrument developed within the laboratory for that purpose, the Negative Symptom Rating Scale (NSRS). In turn, the NSRS is being used to evaluate the association between negative symptoms and impairments in standardized intelligence testing, social intelligence, movement disorders, and other clinical variables.

Methods Employed: Patients entering the research program of the Neuropsychiatry Branch and patients at the New York State Psychiatric Institute are being evaluated using the NSRS. In addition, the Brief Psychiatric Rating Scale is used to evaluate more general psychiatric symptoms. Some patients are also rated using tests of social intelligence and premorbid social functioning.

Testing of a large number of patients has facilitated the study of the internal consistency of the NSRS itself. In addition, correlations have been made between the NSRS and Brief Psychiatric Rating Scale, intelligence tests, social intelligence measures, and movement disorders as assessed using the Abnormal Involuntary Movement Scale (AIMS).

Major Past Findings: The initial step in this project was publication of the NSRS itself, providing a description of the scale and a method for performing ratings using its ten items. The scale was found to be easy to administer, with acceptable inter-rater reliability. In addition, a high correlation was observed between negative symptoms as measured using the NSRS and other previously published measures of negative symptoms. The scale itself was also used in a clinical trial involving the administration of vasopressin to patients with chronic schizophrenia. In that study a modest, but significant, improvement was found in negative symptoms in patients who were given vasopressin.

New Findings: In an attempt to examine the internal consistency of the NSRS, a factor analysis was conducted on data from NSRS evaluations of 121 patients with chronic schizophrenia. The results revealed a strong internal consistency for the scale, with a distribution of the ten NSRS items into two factors. Moreover, defect signs and symptoms as measured by the NSRS appeared to occur independently of positive symptoms in this sample. In addition, when the defect state was examined in relation to the duration of illness in these patients, it appeared that the defect state increased in severity proportional with the duration of illness.

In a second study in which the relationship between defect symptoms and tardive dyskinesia was examined, no correlation was found between defect symptoms and tardive dyskinesia in 55 neuroleptic-treated chronic schizophrenic patients. This study failed to replicate an earlier finding of increased negative symptoms in patients with tardive dyskinesia.

Significance to Mental Health Research: The defect state is a prominent part of the pathology of schizophrenia. The characterization of negative symptoms and an examination of their relationship to other clinical variables is important to our

understanding of the disease as a whole. Moreover the indentification of how social skills develop, are maintained, and become impaired has important implications for the treatment of schizophrenia.

Proposed Course of Project: The data regarding negative symptoms, general intelligence, and social intelligence will be gathered from a larger cohort of patients. These data will in turn be examined in relation to other clinical variables. Ultimately, this study will include correlations of NSRS data with other neuroscientific data gathered from patients in the Neuropsychiatry Branch research program.

Publications:

Iager, A.-C., Kirch, D., Jeste, D., and Wyatt, R.: Defect symptoms and abnormal involuntary movement in schizophrenia. Biol. Psychiatry 21: 751-755, 1986.

Jaeger, A.-C., Kirch, D., and Wyatt, R.: Negative symptom rating scale: Limitations in psychometric and research methodology (the authors reply). Psychiatry Res. 19: 169-170, 1986.

Jaeger, A.-C., Kirch, D., and Schnur, D.: The negative symptom rating scale: Internal consistency and correlations with positive symptoms. Psychiatry Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02257-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and Neuroradiologic Abnormalities in Tardive Dyskinesia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James B. Lohr, M.D., Medical Staff Fellow, Neuropsychiatry Branch, NIMH

Dr. Jean L. Cadet, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying the efficacy of antioxidant therapy for tardive dyskinesia (TD). The treatment involves alpha-tocopherol. Results reveal a significant improvement in movement disorder and a trend toward improvement in psychopathology in schizophrenic and schizoaffective patients with TD after administration of alpha-tocopherol, but no change in neuroleptic-induced parkinsonism with this agent.

GPO 914-918

Project Description:

Objectives: Persistent tardive dyskinesia (TD) following long-term neuroleptic treatment is a growing problem for which there is no satisfactory treatment. We have developed the hypothesis that some cases of TD may result from neuronal damage due to excess free radical production that may occur during catecholamine metabolism. We therefore decided to assess the efficacy of alpha-tocopherol (vitamin E), a potent free radical scavenging agent, on the clinical signs of TD.

The basic theory underlying the use of these medications concerns the formation of free radicals in the brain. Free radicals are highly reactive chemical species that are toxic to cells, including neurons. It is believed that the chronic use of neuroleptic drugs (which block dopamine receptors) may secondarily increase the amount of dopamine in the brain, and that during the metabolism of dopamine, excess free radicals are formed. These free radicals may cause damage to neurons and neuronal membranes in areas of the brain that may cause abnormal movements. Alpha-tocopherol is a free radical scavenger, and may prevent or reverse this damage.

Methods Employed: The treatment study design is double blind/placebo controlled with drug wash-out periods. The maximum dose is 1200 I.U. of alpha-tocopherol per day and the medication is started at a low dose and raised as tolerated. Alpha-tocopherol is virtually non-toxic. Patients are rated regularly for severity of movement disorder and psychopathology. Abnormal movements are rated using a modified version of the Abnormal Involuntary Movement Scale (AIMS) and a modified Simpson-Angus Scale for Parkinsonism (SAS) by two independent raters "blind" to medication status. Psychopathology is rated on the Brief Psychiatric Rating Scale (BPRS). Blood and urine are examined frequently for side-effects of the medications and for research purposes.

Patients are also evaluated at the beginning of the study for their self-perception of movement disorder using a self-assessment questionnaire based on the AIMS.

Over the past six months, 15 patients with the diagnosis of either chronic schizophrenia, or schizoaffective disorder (by DMS-III) criteria) or persistent TD or tardive dyskinesia for at least two years completed the study on alpha-tocopherol. TD was diagnosed by two independent raters using specific criteria. Patients received alpha-tocopherol and matched placebo in a randomized cross-over design. The dose of alpha-tocopherol was raised from 400 I.U. p.o. q.d. to 400 I.U. p.o. t.i.d. over a two week period and maintained at that level for an additional two weeks.

New Findings: Patients demonstrated a significant overall reduction in AIMS score after treatment with alpha-tocopherol, but not after placebo (7.5 ± 4.8 vs 13.5 ± 6.4, two-tailed $t=6.39$, $p<0.001$). The mean reduction in the AIMS score with alpha-tocopherol was 43%, with 7 patients showing greater than 50% reduction in their dyskinesia. There was no difference in SAS score after alpha-tocopherol compared to placebo (5.3 ± 5.5 vs 4.5 ± 4.7, NS). In a subset of 10 patients who had blood drawn for alpha-tocopherol concentrations, the mean serum alpha-tocopherol level rose from 11.8 ± 4.8 mcg/ml to 23.7 mcg/ml (normal range, 5-20 mcg/ml).

There was a trend for a decrease in BPRS score after alpha-tocopherol (22.7 ± 18.9 vs 16.8 ± 17.5, two-tailed $t=2.13$, NS). However, when the BPRS score was divided

according to subscores (anxiety, depression agression, mania, positive or productive symptoms, and negative or withdrawal symptoms), the alpha-tocopherol score was significantly less than the placebo score on the anxiety subscale (1.1±1.9 vs 2.4±2.5, t=3.48, p<0.005) and the depression subscale (1.4±2.4 vs 3.1±3.2, t=3.39, p<0.005).

A subset of 12 patients completed a second placebo and active treatment phase. Again, there was a significant overall reduction in AIMS score after treatment with alpha-tocopherol but not placebo (6.2±3.3 vs 11.1±5.1, two-tailed t=4.27, p<0.005), but no difference in SAS score (3.7±4.8 vs 4.1±4.0, NS). There was no significant difference between mean AIMS scores after the two placebo periods (13.5±6.4 vs 11.1±5.1, NS).

Patients were then divided into two groups according to whether they showed a greater than 50% improvement after alpha-tocopherol (n=7) or less than 50% improvement (n=8). The group with greater than 50% improvement was noted to have a significantly shorter duration of TD (1.13±0.35 vs 3.29±2.06 years, two-tailed t=2.94, df=13, p<0.02), and a significantly later age of onset of psychiatric illness (27.1±7.3 vs 19.7±1.7 years, two-tailed t=2.6, df=13, p<0.03) than the group with less than 50% improvement. These groups did not differ in terms of other clinical variables.

Significance to Mental Health Research: Neuroleptic-induced TD is a major public health concern in psychopharmacology. With a reported prevalence of over 25% among chronic inpatients, it is an important limiting factor in the use of neuroleptics in psychiatry. It is possible that, in part, TD represents a syndrome of neuroleptic-induced brain damage which may be partially reversible or even preventable with agents that scavenge free radicals or chelate transitional metals.

Proposed Course of Project: We plan to extend the study using a higher dose of alpha-tocopherol over a prolonged period of time over the next two years.

Publications:

Lohr, J.B., Wisniewski, A., and Jeste, D.V.: Neurological aspects of tardive dyskinesia. In Nasrallah, H., and Weinberger, D.R. (Eds.): Handbook of Schizophrenia, Vol. 1: Neurology of Schizophrenia. Amsterdam, Elsevier Science Publishers, 1986, pp. 97-119.

Cadet, J.L., and Lohr, J.B.: Free radicals and the developmental pathobiology of schizophrenic burnout. Integr. Psychiatry 5: 40-48, 1987.

Lohr, J.B., Lohr, M.A., Wasli, E., Hilliard, B., Larson, L., Vardiman, E., Wade, L., and Jeste, D.V.: Self-perception of tardive dyskinesia and neuroleptic-induced parkinsonism: A study of clinical correlates. Psychopharm. Bull. 23: 211-214, 1987.

Lohr, J.B., Cadet, J.L., Lohr, M.A., Jeste, D.V., and Wyatt, R.J.: Alpha-tocopherol in tardive dyskinesia. Lancet 1: 913-914, 1987.

Jeste, D.V., Lohr, J.B., Cadet, J.L., and Wyatt, R.J.: Tardive dyskinesia: Neurological mechanisms. In Shagass, C. (Ed.): Biological Psychiatry 1985:

Proceedings of the IVth World Congress of Biological Psychiatry. New York, Elsevier Science Publishers, in press.

Jeste, D.V., Lohr, J.B., Kaufmann, C.A., and Wyatt, R.J.: Pathophysiology of tardive dyskinesia: Evaluation of supersensitivity theory and alternative hypotheses. In Casey, D.E., and Gardos, G. (Eds.): Neuroleptics and Tardive Dyskinesia: From Dogma to Reason. Washington, D.C., American Psychiatric Press, in press.

Cadet, J.L., Lohr, J.B., and Jeste, D.V.: Tardive dyskinesia and schizophrenic burnout: The possible involvement of cytotoxic free radicals. In Henn, F.A., and DeLisi, L. (Eds.): Handbook of Schizophrenia, Vol. 3. Amsterdam, Elsevier Science Publishers, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02258-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Quantitative Neuropathology of Aging and Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James B. Lohr, M.D., Medical Staff Fellow, NPB, IRP, NIMH

Dr. Dilip Jeste, Department of Psychiatry and Neurosciences, University of California at San Diego; Dr. Joseph Parisi, Chairman, Department of Neuropathology, Armed Forces Institute of Pathology, Bethesda, Maryland; Dr. Francine Benes, Department of Psychiatry, McLean Research Center, Boston, MA.

COOPERATING UNITS *(if any)*

Dept. Psychiat. Neurosci., Univ. of California at San Diego; Dept. Neurol., George Washington Univ. Hosp., Washington, D.C.; Dept. Neuropathol., Armed Forces Inst. Pathol., Bethesda, MD; Dept. Psychiatry, McLean Research Center, Boston, MA

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying quantitative volume, neuronal density, neuronal size and nuclear size in selected areas of brains from patients with certain neuropsychiatric disorders as well as normal controls from different age groups. Results to date indicate that there is normally age-related Purkinje cell loss in the cerebellum, pyramidal cell loss in area CA4 of the hippocampus, and neuron loss in the locus ceruleus. In schizophrenic subjects, we found a significant decrease in the pyramidal cell density in area CA4 of the hippocampus, mainly in the left hemisphere when anteriorly compared to controls. We found no significant morphological differences between schizophrenic patients, affective disorder patients and normal controls in cerebellum. There was a trend toward a reduction in the volume of the locus ceruleus in schizophrenics, without a loss of neurons.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: The neuron forms the basic structural and functional unit of the nervous system. Neuronal changes are usually at the core of most gray-matter diseases of the brain. Indirect evidence implicates neuronal damage in specific areas of the brain in normal aging as well as in a number of neuropsychiatric disorders. Earlier studies of the neuropathology of schizophrenia and major affective disorders have often yielded conflicting results. One reason for this lack of uniform findings is the use of qualitative methods for studying neuropathology.

We rely on the newly developed computerized neuronal imaging systems that provide objective and quantitative data. The main goals of our studies are to obtain such data in selected regions of the brain -- e.g., the limbic system and cerebellum in schizophrenia and affective disorders, hippocampus in dementias, basal ganglia in movement disorders. The studies are designed to answer specific questions: e.g., Is there a selective vulnerability to neuron loss of different areas of the brain with age? Are morphological abnormalities seen in the limbic system of schizophrenic patients? Does long-term neuroleptic treatment have a cytotoxic effect on certain neurons? Do unipolar and bipolar affective disorder patients differ significantly in their neuropathologic lesions? Such information will have not only theoretical, but also potentially therapeutic implications.

For our studies we have access to one of the finest neuropathology collections in the world - viz., the Yakovlev collection at the Armed Forces Institute of Pathology. This collection is well known for its high level of uniformity of brain sectioning and processing. According to the forward to the catalogue of the Yakovlev collection by Denny-Brown, "There is no other collection of neuroanatomical material in serial sections consistently fixed, embedded, cut, stained and mounted to such exacting standards," (Yakovlev, P.E., 1972, unpublished).

Methods Employed: The material came from the Yakovlev collection at the Armed Forces Institute of Pathology, Washington, D.C. In this collection, all the brains were fixed in formalin and embedded in celloidin, and 35 um-thick sections were made. Every 20th section was stained with cresyl violet. We selected coronal, sagittal and horizontal sections through right and left hippocampi and right and left loci cerulei. Hippocampal measurements were made in 23 subjects in the "normative" series (i.e., subjects without documented neurological abnormality prior to death), 13 subjects with schizophrenic illness, and 10 leucotomized control subjects with diagnoses of affective disorders, other psychiatric disorders, or chronic pain. Locus ceruleus measurements were made in 13 normative subjects, 15 schizophrenic subjects, and 11 leucotomized control subjects. Because of Dr. Yakovlev's interest in prefrontal leucotomy, all schizophrenic subjects had undergone this procedure.

Selection of sections: Specific sections were selected according to specified atlas criteria.

Apparatus: A Zeiss Videoplan computing planimeter was used for the measurements. The whole apparatus consisted of the Videoplan attached to a microscope through a television monitor, with the image under the microscope being displayed on the monitor. By moving an electronic mouse across a magnetic tablet, images on the

monitor could be outlined or counted, with automatic computation of area and cell density.

Measurements: Hippocampus--The four hippocampal sectors (CA1 through CA4) were delineated according to the descriptions of Lorente de No and Braak. We made several morphological assessments in each hippocampal sector. The measurements included pyramidal cell areal density in both anterior and posterior hippocampus, and sectorial volume. Locus ceruleus--We measured the volume and total number of neurons in the locus ceruleus as well as the neuronal cross-sectional area and the percentage of neuronal area occupied by neuromelanin.

Reliability: All the morphometric measurements were done "blind" to age and other clinical data on the subjects. The intraclass correlation coefficient for each parameter measured by three independent raters was at least 0.82 ($p<0.0001$).

Major Past Findings:
(1) Cerebellum and aging. We studied 49 cerebella from the normative series of the Yakovlev collection. We found a highly significant negative correlation between age and Purkinje cell density, especially in the anterior vermis ($r=.62$, $p<0.0001$). No correlation with age was found in Purkinje cell area or Purkinje cell nucleus area, nor with dentate nucleus multipolar cell area. No differences were observed between men (n=30) and women (n=19).
(2) Cerebellum and disorders of the basal ganglia. We found a significant (about 50%) loss of Purkinje cells in eight out of 17 patients with Huntington's disease. Patients with Parkinson's disease exhibited a less consistent loss of Purkinje cells. These findings are consistent with our clinical studies showing signs of cerebellar dysfunction in patients with Huntington's and Parkinson's disease.
(3) Cerebellum and psychiatric disorders. From the Yakovlev collection, we examined 23 brains from schizophrenic subjects, and 23 brains from leucotomized controls (seven manic-depressive, five unipolar depressive, three psychopathy, one anorexia nervosa, one psychogenic pain disorder, and seven with chronic pain). We compared these groups with an age- and sex-matched group of 37 brains from the normative series for Purkinje cell density in anterior and posterior vermis, cerebellar hemispheres and cerebellar Purkinje cell area and nuclear area, and dentate multipolar cell density. No significant differences were found between the groups on these measures.
(4) Hippocampus and aging. We studied right and left hippocampi from 23 normative brains in the Yakovlev collection, age range 4-94. We found an age-related loss of pyramidal cells in all four sectors, CA1-CA4, but this only reached significance for CA4. Also, we found an age-related decrease in volume which was most significant for sectors CA3 and CA4. No significant change in neuronal or nuclear cross-sectional area with age was found.
(5) Hippocampus and schizophrenia. There appears to be a loss of pyramidal cells in sector CA4 in the anterior hippocampus in schizophrenia, most significant in the left hemisphere. Although, in general, schizophrenic patients have a lower pyramidal cell density than normals or leucotomized controls, no significant differences were observed in other hippocampal sectors (CA1-CA3) or in the posterior hippocampus. Of the three brain areas we have studied so far in schizophrenia, this loss of pyramidal cells in anterior sector CA4 represents the only markedly abnormal finding. This suggests that morphological changes in schizophrenia may be quite subtle, and may be relatively specific to certain areas of the brain.

New Findings: The locus ceruleus (LC), a pigmented brainstem nucleus rich in noradrenergic neurons, has been proposed to be involved in the pathophysiology of aging and schizophrenia. We undertook a quantitative neuropathological study of the LC in these two conditions. A computing planimeter was employed to count the total number of neurons and measure the volume of the LC, neuronal cross-sectional area, and percent of neuronal area occupied by neuromelanin in the brains of 39 subjects; 13 "normative," 15 leucotomized schizophrenics (most had died in the preneuroleptic era), and 11 leucotomized non-schizophrenic control patients, ranging in age from 11 to 94 years. There was a significant inverse correlation between age and total number of LC neurons, neuronal size, and LC volume, and a significant positive correlation between age and the percentage of neuronal area occupied by neuromelanin. Although schizophrenics did not differ significantly from control groups on any of the parameters of LC morphology, there was a trend for reduced LC volume in schizophrenic brains. Also, the LC of leucotomized patients tended to have increased neuromelanin content and slightly increased cell counts compared to normals, although the importance of this finding is not clear.

Significance to Mental Health Research: Neuropathology is one of the most neglected areas in mental health research, and this is even more true of quantitative neuropathology. The latter is important if we are to define the "sites of lesion" in disorders such as schizophrenia and major affective disorders, and to understand the process of aging better.

Such studies will improve insight into the pathophysiology of aging and various neuropsychiatric disorders, and further, lead to development of treatments that may have actions on specific brain regions. From another prespective, such studies may also highlight neurotoxicity of currently available treatments. Quantitative neuropathology will also help in brain studies of animal models of aging and certain neuropsychiatric disorders.

Proposed Course of Project: This work began three years ago on a small scale. We will be expanding the work to include finer measurements of more brain areas in the next year.

Publications:

Mani, R.B., Lohr, J.B., Ludwig, C., and Jeste, D.V.: Hippocampal pyramidal cells and aging in the human: A quantitative neuronometric study of individual sectors of the hippocampus. Exp. Neurology 94: 29-40, 1986.

Lohr, J.B., and Jeste, D.V.: Cerebellar pathology in schizophrenia: A review and a quantitative neuronometric study. Biol. Psychiatry 21: 865-875, 1986.

Jeste, D.V., Lohr, J.B., Mani, R., Cadet, J.L., and Ludwig, C.: Neuronal loss in normal aging and neuropsychiatric dementias. In Jeste, D.V. (Ed.): Neuropsychiatric Dementias: Current Perspectives. Washington, D.C., American Psychiatric Press, 1986, pp. 111-143.

Jeste, D.V., Lohr, J.B., and Mani, R.: Quantitative neuropathology and aging. In Shagass, C. (Ed.): Biological Psychiatry 1985: Proceedings of the IVth World Congress of Biological Psychiatry. New York, Elsevier Science Publishers, in press.

Lohr, J.B., and Jeste, D.V.: Neuronometric studies of cerebellum and hippocampus in major psychiatric disorders. In Shagass, C. (Ed.): Biological Psychiatry 1985: Proceedings of the IVth World Congress of Biological Psychiatry. New York, Elsevier Science Publishers, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02259-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Peripheral and Central Catecholamine Turnover in Mental Illnesses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

Farouk Karoum, Ph.D., Chemist, Neuropsychiatry Branch, IRP, NIMH

Dr. Esa Korpi, Research Laboratories, Helsinki, Finland; Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH; Dr. William B. Lawson, Faculty Member, University of California, Irvine, California; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Markku Linnoila, LCS, DICBR, NIAAA; Dr. Alan J. Zametkin, Laboratory of Clinical Science, IRP, NIMH

COOPERATING UNITS *(if any)*

Research Laboratories, Helsinki, Finland; University of California, Irvine, California; Laboratory of Clinical Studies, NIAAA; Laboratory of Clinical Sciences, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Psychopharmacology

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.33	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Combined gas chromatographic mass spectrometric methods previously developed for the assay of biogenic amines in various biological media have been employed to assess total body turnover of norepinephrine (sum NE) and dopamine (sum DA) in both human subjects and rats. We have also compared changes in sum NE and sum DA after a number of pharmacological manimpulations in rats. The aim of these animal studies was to gain an insight into how these pharmacological treatments influence brain catecholamines in depression, schizophrenia and hyperactive children.

(1) Consistent with our 1985 and 1986 Annual Reports, we have continued to gather additional supportive data that suggest a tendency for sum NE to be elevated in major depression. We have also observed a positive correlation between urinary-free cortico and urinary NE and VMA.

(2) Total body NE and DA turnover were assessed in both hyperactive children and adults after a number of pharmacological manipulations. The results indicated a correlation between therapeutic benefit and changes in both sum NE and DA irrespective of the direction of change.

(3) The effects of four commonly used antidepressant treatments on rat peripheral and central catecholamines were evaluated. A good correlation between the effects of these drugs and sum NE and sum DA in humans and rats was observed. It is suggested that because of this correlation, changes in the rat brain amines observed probably resemble the changes these treatments induce in the human brain. The four treatments employed were chronic zimelidine, desipramine, electroconvulsion and lithium.

(4) We are currently attempting to reproduce our initial study on DA and NE turnover in schizophrenia and hope to also include patients with tardive dyskinesia.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: To assess and determine the role of peripheral and central catecholamine in mental illnesses.

Methods Employed: All biochemical analyses were performed by combined gas chromatographic mass spectrometric methods developed in this laboratory. In some studies rats with unilaterally lesioned substantia nigra with 6-hydroxydopamine were used.

Major Findings: The abilities of five types of antidepressants commonly employed in the management of depression to reduce sum NE in both humans and rats and our previous report (1985 Annual Report) of increased sum NE in depressed patients with melancholia suggest that depression is associated with a state of hyperactive peripheral noradrenergic condition. Furthermore, since in rats changes in sum NE were found to be parallel to similar changes in brain NE turnover, it could be argued that the above five antidepressants (DMI, ZMI, ECT, Li and low doses of clorgyline) also reduce brain NE turnover in humans. This latter view is consistent with current belief that antidepressants down regulate noradrenergic receptors.

In contrast to depressed patients who tend to exhibit less consistent association between the effects of antidepressants and sum DA, in schizophrenia sum DA is the parameter that is abnormal. Thus, while sum NE in chronic schizophrenic patients appeared normal, sum DA was found to be low when compared to age and gender matched controls. The reduction in sum DA observed in medication free chronic schizophrenic patients becomes clearer when sum DA is expressed in terms of the ratio of sum DA/sum NE. Haloperidol treatment of chronic schizophrenics normalized this ratio. The results of our investigation raise the possibility that schizophrenia may be associated with an imbalance between noradrenergic and dopaminergic activities and that neuroleptic medication preferentially stimulates DA turnover thereby balancing the activity between the two amines.

Our failure to find consistent changes in sum NE or sum DA in hyperactive children after administration of a variety of medications which have proven therapeutic benefits raised certain issues. The first is that catecholamines may not be involved in the etiology of the disorder. The second, in contrast, may be related to a possibility that the induction of change in peripheral and/or central noradrenergic systems, irrespective of the direction of change, is all that is needed to calm these children and improve their attention span.

Significance to Mental Health Research: Our findings, which are related to total body turnover of catecholamines in depression, schizophrenia and hyperactivity in children, have convinced us that the methods employed are useful in studying the role of catecholamines in mental illnesses.

Proposed Course of Project: We plan to expand our investigation to include other antidepressants. We also plan to continue to use rats to correlate total body amine turnovers with central amine turnover and metabolism. Since we already have methods that can accurately and reliably measure a variety of biogenic amines in biological materials, we will include, whenever necessary, information on the disposition of such amines as phenylethylamine, tyramine, and the indoleamines.

Emphasis in all future investigations will be directed towards understanding how neuroleptics influence both central and peripheral dopaminergic systems. In these studies we plan to carry out simultaneous measurements of sum DA and sum NE as well as plasma and CSF concentrations of catecholamines and their metabolites in schizophrenics while on and off neuroleptics.

We are currently collecting more urines from schizophrenic patients both on and off medication. We are also collecting urines from patients with tardive dyskinesia.

Publications:

Roy, A., Linnoila, M., Karoum, F., and Pickar, D.: Urinary-free cortisol in depressed patients and controls: Relationship to urinary indices of noradrenergic function. Psychol. Med., in press.

Roy, A., Karoum, F., Linnoila, M., and Pickar, D.: Thyrotropin-releasing hormone test in unipolar depressed patients and controls: Relationship to clinical and biological variables. Acta Psychol. Scand., in press.

Karoum, F., Karson, C.N., Bigelow, L.B., Lawson, W.B., and Wyatt, R.J.: Preliminary evidence of reduced combined output of dopamine and its metabolites in chronic schizophrenia. Arch. Gen. Psychiat., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02261-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Phenomena in Schizophrenia: Quantification in an Effort to Subtype

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH

Dr. Llewellyn B. Bigelow, Associate Clinical Director, WAW Division, Saint Elizabeths Hospital, NIMH; Dr. Darrell G. Kirch, Senior Staff Fellow, NPB, DIRP, NIMH; Dr. Joel E. Kleinman, Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- [XX] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02262-03 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electroretinography in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Myles Jay Jaffe, O.D. Ph.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Craig N. Karson, Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The ganzfeld electroretinogram has revealed that patients with chronic Parkinson's disease have a diminished sensitivity of their retina to light; this defect can, in part, be restored by an i.v. infusion of L-dopa. Together, these findings indicate that the retina is another CNS structure that is involved in Parkinson's disease. In addition, morphine modulates the sensitivity of the retina to light. In combination with other studies, we have found that the actions of morphine may be related to the action of dopamine within the retina.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: Using neuropharmacological agents and the lesions presumed to be inherent in Parkinson's disease, we have better evaluated how the retina can be used for objective study of CNS function in humans.

Methods Employed: Collected data was derived from recordings made with the ganzfeld electroretinogram (ERG), a non-invasive tool that records primarily from the rod and cone cells within the photoreceptor layer of the retina. The effects of morphine were studied in normal volunteers: they received a baseline ERG, and then 10 mg of morphine (i.m.) followed by a second ERG. L-dopa was evaluated in patients with chronic Parkinson's disease who had been given a drug-holiday from dopaminergic agonists for about 48 hr; following this, a baseline ERG was recorded. These patients were then started on an i.v. infusion of L-dopa and allowed to stabilize; they then received a second ERG.

Major Past Findings: Increased latency of the blue-sensitive cone ERG has been observed in patients with schizophrenia off-medication. In addition, specific dopamine blockers and GABA potentiators have been shown to have an attenuating effect on retinal sensitivity.

New Findings: Consistent with the ideas of others, Parkinson's disease appears to be a disease that affects dopaminergic areas of the CNS in addition to the basal ganglia. Physiological levels of dopamine seem to be required for the retina to react normally to light. If these levels decrease, retinal responsivity decreases as well. When L-dopa is administered i.v., retinal responsivity has been shown to improve in the direction of normal.

In addition, morphine also appears to affect the gain control of the retina's sensitivity to light. Latency has been shown to be delayed by morphine: both the rods and all three classes of cones have been shown to be altered. Our data shows how morphine attenuates the ERG and provides evidence for a presynaptic mechanism by which opiates modulate dopaminergic cells of the retina. It also supports findings by others that morphine results in a concentration-dependent decrease in the stimulation-evoked release of [^{3}H]dopamine from rabbit retina. We postulate that, although the opiate receptors are different from the inhibitory dopamine autoreceptor, morphine, upon binding to presynaptic dopamine neurons, attenuates the cellular processes that generate the rod b-wave and slows the clock that times the generation of the oscillatory potentials.

Significance to Mental Health Research: Retinal involvement in patients with Parkinson's disease indicates that CNS dopamine is affected in regions outside of the basal ganglia. Future explanations for the pathogenesis of Parkinson's disease should also include a rationale for our data. Our studies with morphine suggest an inter-relationship between the dopamine receptor and the morphine receptor in the human retina and imply that this may exist elsewhere in the brain.

Proposed Course of Project: We plan to extend these findings into the neuropharmacological actions of similar compounds on the ganglion cell layer of the retina via the Pattern ERG.

Publications:

Higgins, K.E., Meyers, S.M., Jaffe, M.J., Roy, M.S., and de Monasterio, F.M.: Temporary loss of foveal contrast sensitivity associated with pan retinal photocoagulation. Arch. Ophthal. 104: 997-1003, 1986.

Jaffe, M.J., Caruso, R.C., de Monasterio, F.M., and Nussenblatt, R.: Chronic eyelid closure and its effect upon the human cone and rod electroretinogram. Am. J. Optom. Physiol. Optics, in press.

Jaffe, M.J., Karson, C.N., Roy, A., and de Monasterio, F.M.: Schizophrenia: Alterations in blue-cone function. Psychiatry Res., in press.

Jaffe, M.J., Bruno, G., Campbell, G., Lavine, R., Karson, C.N., and Weinberger, D.R.: Ganzfeld electroretinographic findings in Parkinsonism: Untreated patients and the effect of L-dopa intravenous infusion. J. Neurol. Neurosurg. Psychiat., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02263-03 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Haloperidol Pharmacodynamics and Clinical Response in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Darrell G. Kirch, M.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Llewellyn B. Bigelow, Associate Clinical Director for the William A. White Division, Saint Elizabeths Hospital; Dr. Gregory M. Straw, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Markku Linnoila, NIAAA; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Greg Gerhardt, University of Colorado Health Sciences Center; Dr. Robert Freedman, University of Colorado Health Sciences Center

COOPERATING UNITS *(if any)*

NIAAA; University of Colorado Health Sciences Center

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of a standardized research sequence in the NIMH intramural clinical program located at Saint Elizabeths, patients with schizophrenia are withdrawn from neuroleptic medication and then (after clinical relapse) treated with a fixed dose of haloperidol. This has in turn allowed initiation of a variety of studies regarding the pharmacokinetics of haloperidol. Other pharmacological issues examined in these patients are drug-drug interactions (specifically involving haloperidol and a number of other drugs, including nicotine, caffeine, retinoic acids, and dopaminergic agonists). In addition, basic science investigations regarding these drugs are being conducted.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description:

Objectives: This project makes use of a high performance liquid chromatography method for the measurement of haloperidol and its reduced metabolite in serum, red blood cells, and tissue samples. Specific problems being studied include the relationship between serum concentrations and clinical variables, pharmacokinetic phenomena, and drug-drug interactions. Other problems being examined include the effects of haloperidol, nicotine, and caffeine on catecholamines. Research is also being conducted regarding the impact of nicotine and caffeine use on patients with chronic schizophrenia.

Methods Employed: The method of quantification of haloperidol and reduced haloperidol involves liquid chromatography after a liquid-liquid extraction process, as previously reported. Research subjects who come into the Neuropsychiatry Branch clinical research program are stabilized on the neuroleptic they are taking when they arrive. After a thorough psychiatric and medical examination, they are placed on coded neuroleptics. In this way, they are treated with both active fixed-dose haloperidol (0.4 mg/kg/day) or placebo in double-blind fashion. This allows both pharmacokinetic and steady state samples to be collected for serum haloperidol concentrations. Moreover, haloperidol can be measured while the patients are being treated with other drugs, including retinoic acids. In addition, blood samples can be collected for quantification of nicotine, cotinine, and caffeine.

Basic science studies are being conducted using the administration of haloperidol by injection and the chronic infusion of other drugs via osmotic mini-pumps.

Major Past Findings: Previous single cell recordings in rats have shown that the metabolite of haloperidol, reduced haloperidol, appears to be inactive. Moreover, studies of the relationship between serum haloperidol concentration and clinical response indicate that above a threshold concentration (which appears to be approximately 5 ng/ml) patients have the same degree of clinical response regardless of how high a serum concentration is attained. Thus, our own data have failed to show a "therapeutic window" for haloperidol as has been observed by other investigators.

Pharmacokinetic data have been accumulated in an ongoing fashion regarding the response to an acute dose of haloperidol and the washout from haloperidol after withdrawal of the drug. Initial data analyses showed a peak in serum concentration 3 to 5 hours after acute administration with a significant correlation between this acute response and ultimate steady state concentration. Withdrawal data have shown an initial half life of less than 24 hours with a slower later phase of elimination of the drug. Smoking has been revealed to lower serum haloperidol concentrations.

Assessment of plasma monoamine concentrations in patients before and after withdrawal from neuroleptic have shown an increase in both homovanillic acid and 3-methoxy-4-hydroxyphenylglycol in patients after they were withdrawn from neuroleptics. Only the increase in the latter compound was statistically significant.

Research involving nicotine in the past showed that patients with the diagnosis of schizophrenia are more likely to smoke than chronically hospitalized patients with other diagnoses.

New Findings: An attempt has been made to replicate the earlier findings regarding the relationship between serum concentrations and clinical response. This has involved a multi-center study being conducted by the World Health Organization in which patients are given a fixed high or low dose of haloperidol, serum concentrations are measured, and clinical response is assessed. Initial analyses of the data regarding clinical response at 4 weeks again showed that above a certain threshold, patients appear to have a comparable response and there was no evidence of a "therapeutic window." Data from more subjects is being examined for the WHO study.

Data regarding the interaction of haloperidol and retinoic acids in clinical studies and animal models has been gathered and is being reported under a separate project title.

With regard to the effects of nicotine, a larger epidemiologic survey has been conducted. The finding of increased smoking among patients with schizophrenia compared with other diagnoses has been supported. Moreover, it appears that smokers who have schizophrenia are more likely to also have tardive dyskinesia than those who do not smoke. Animal research regarding nicotine has confirmed that there is a decrease in dopamine turnover in striatum, frontal cortex, and hypothalamus when rats are chronically treated with nicotine for a period of three weeks.

Significance to Mental Health Research: Haloperidol remains one of the most commonly used drugs in the treatment of schizophrenia. The data produced by this laboratory may help provide a more rational strategy for dosages. The studies regarding nicotine and caffeine not only may increase our understanding of why patients with schizophrenia are so prone to use these drugs, but also may reveal some information regarding the mechanisms underlying dependence on these substances in normal subjects. The studies of catecholamines are directed at clarifying the basic central nervous system neurochemistry involved in schizophrenia.

Proposed Course of Project: Pharmacokinetic studies of haloperidol at this point will focus on the ongoing World Health Organization study as described above. Both clinical and basic science studies directed at elucidating the mechanisms of nicotine and caffeine use will be continued.

Publications:

Iager, A.-C., Kirch, D., Bigelow, L., and Karson, C.: Treatment of schizophrenia with a vasopressin analogue. Am. J. Psychiatry 143: 375-377, 1986.

Potkin, S., Urbancheck, M., Kirch, D., and Bunney, W.E., Jr.: Evaluation of optimal doses of a neuroleptic: Preliminary results. Clin. Neuropharmacol. 9 (Suppl. 4): 437-439, 1986.

Kirch, D.: Pharmacology and behavior. In Weiner, J. (Ed.): Behavioral Sciences. New York, Wiley Press, in press.

Kirch, D.: Laboratory tests in psychiatry. In Kaplan, H. and Sadock, B. (Eds.): The Comprehensive Textbook of Psychiatry, 5th Edition. Baltimore, Williams and Wilkins, in press.

Kirch, D., Gerhardt, G., Shelton, R., Freedman, R., and Wyatt, R.: The effect of chronic nicotine administration on monoamine and monoamine metabolite concentrations in rat brain. Clin. Neuropharmacol., in press.

Kirch, D., Bigelow, L., Korpi, E., Wagner, R., Zalcman, S., and Wyatt, R.: Serum haloperidol concentration and clinical response in schizophrenia. Schiz. Bull., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02264-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Post Mortem Brain Tissue Examination in Psychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joel E. Kleinman, M.D., Ph.D., Chief, Section on Clinical Brain Studies, NPB, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Craig N.Karson Staff Psychiatrist, NPB, IRP, NIMH; Dr. Esa Korpi, State Alcohol Monopoly, Helsinki Finland; Dr. Markku Linnoila, NIAAA, NIMH; Dr. Farouk Karoum, Chemist, NPB, IRP, NIMH; Dr. Daniel Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. Anita Feenstra, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

State Alcohol Monopoly, Helsinki, Finland; NIAAA; Johns Hopkins University; Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Brain Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02267-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Brain Electrical Activity Mapping in Neuropsychiatric Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Craig N. Karson, Staff Psychiatrist, NPB, IRP, NIMH

Dr. Terry Goldberg, Special Expert, NPB, IRP, NIMH; Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Ralph Fawcett, Medical Staff Fellow, NPB, IRP, NIMH; Dr. Richard Coppola, Sr. Engineer Officer, NPB, IRP, NIMH; Dr. Daniel R. Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02268-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Clinical Phenomenology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Frank W. Putnam, M.D., Staff Psychiatrist, Neuropsychiatry Branch, IRP, NIMH

Dr. E.A. Silberman, USUHS; Dr. Robert Post, Biological Psychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

USUHS
Biological Psychiatry Branch, IRP, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project was transferred to the Laboratory of Developmental Psychology in June 1986.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02269-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Development, Reliability and Validity of a Dissociation Scale

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Frank W. Putnam, M.D., Staff Psychiatrist, Neuropsychiatry Branch, IRP, NIMH

Dr. Eve Bernstein, Department of Psychology, American University

COOPERATING UNITS *(if any)*

Department of Psychology, American University

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was transferred to the Laboratory of Developmental Psychology in June 1986.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02270-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

The Psychophysiology of Multiple Personality

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Frank W. Putnam, M.D., Staff Psychiatrist, Neuropsychiatry Branch, IRP, NIMH

Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Daniel Weinberger, Chief, Clinical Neuropsychiatry Section, NPB, IRP, NIMH; Dr. Richard Coppola, Engineer, Clinical Neuropsychiatry Section, NPB, IRP, NIMH; Dr. Robert M. Post, Biological Psychiatry Branch, IRP, NIMH; Dr. Theodore Zahn, Laboratory of Psychology and Psychopathology, BPB, NIMH

COOPERATING UNITS *(if any)*

Biological Psychiatry Branch, IRP, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

This project was transferred to the Laboratory of Developmental Psychology in June 1986.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02273-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

White House Cases: Predictors of Future Violence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

David Shore, M.D., Staff Psychiatrist, NPB, IRP, NIMH

C. Richard Filson, Ed.D., Richardson Division, SEH; Kenneth Baker, Behavioral Research Section, U.S. Secret Service; Dr. Charles Kinderman, Bureau of Justice Statistics, U.S. Department of Justice; Ken Candell, Uniform Crime Reporting Division; William Garvie, Identification Division, FBI

COOPERATING UNITS *(if any)*

Richardson Div., St. Elizabeths Hosp., Wash. D.C.; Behavioral Research Sect., Intelligence Div., U.S. Secret Service; Bureau of Justice Statistics, U.S. Department of Justice; Uniform Crime Reporting Div. and Identification Div., FBI

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS.	PROFESSIONAL.	OTHER.
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided)*

This project has been completed and terminated.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02274-03 NPB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Exploration of New Methods for Treatment of Intractable Epilepsy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Janice Stevens, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH
NINCDS, Bethesda, Maryland

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to devise more effective methods for treatment of epilepsy that is intractable by conventional modern medical therapy or surgical intervention, we explored the feasibility of brain grafts of GABAergic brain tissue to specific brain areas in rat models of epilepsy. We worked with two satisfactory experimental epilepsy models in the laboratory: (1) audiogenic seizures in genetically predisposed rats and (2) amygdala kindled rats.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

1) Rats with audiogenic seizures (obtained from Dr. Phillip Jobe) were tested for latency and type of seizures for four successive weekly intervals after which nine animals with stable seizure type were implanted with fetal cerebellar tissue from rats of 15-day gestation. The tissue was placed over the inferior colliculus bilaterally after removal of the occipital poles by suction through burrholes. Viable cerebellar grafts grew on top of superior colliculus in one animal but were encapsulated and remained separate from the host brain tissue. There was no change in seizure threshold in the grafted animals. Injury to the inferior colliculus by the suction procedure occurred in two animals and was associated with elevation of seizure threshold. A second series of 6 rats was implanted one month ago with adrenal tissue in lateral ventricle bilaterally. Only slight change has been observed in convulsive threshold to date.

2) Twenty Sprague Dawley rats were kindled to Stage V seizures after which they were implanted in endopiriform area bilaterally with 15 day gestation fetal cerebellar or cortical tissue. Three animals improved (kindling threshold rose by 2 times or more) transiently between the third and seventh week post-grafting. All reverted to previous seizure threshold thereafter. Pathological examination indicated that grafts grew well intraparenchymally but were in a majority of cases dorsal to endopiriform area. Cortical grafts were equally successful as cerebellar grafts. Glutamic acid decarboxylase (GAD) immunohistochemistry, a good marker in our hands for the enzyme in adult cerebellum, was not very successful in staining grafted tissues.

Significance: These are the first trials using brain transplants in the search for a new treatment for epilepsy. Although these experiments have not proven very successful, we have learned a good deal that should improve success with future attempts. Above all we learned that undivided transplanted cerebellar tissue remains in an encapsulated tumor-like mass when implanted in brain or ventricle and probably would not be a satisfactory source of GABAergic cells for transplant. Cortex with its 59% GABA cells or dissociated Purkinje cells may be more satisfactory.

Significance to Mental Health Research: Epilepsy is one of the most prevalent and most disabling neuropsychiatric problems in the United States, and indeed in the world. Epilepsy affects approximately 0.5 percent of the U.S. population. It is young people who are most affected, and 20 to 25 percent of those affected are severely and permanently handicapped despite enlightened use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required for this disorder.

Proposed Course of Project: Our immediate plans for this project are as follows: We have completed pathologic studies on the first 3 groups of epileptic rats and are continuing to test the 4th group (audiogenics) who are now 1 month post adrenal ventricular grafts. Tests will continue through 8 weeks post transplant, after which the animals will be sacrificed and a decision made whether to do more animals.

Publications:

Stevens, J.R.: Neuropathological findings in schizophrenia. In See, W. and Lee, C. (Eds.): Transmitters and Ligands in Psychiatry, Livingston and Churchill, in press.

Stevens, J.R.: Symptoms of limbic dysfunction in the acute psychoses of Zimbabwe. Int. J. Neurology, in press.

Stevens, J.R.: Brief psychoses: Do they contribute to the good prognosis and equal prevalence of schizophrenia in developing countries? Brit. J. Psychiatry, in press.

Stevens, J.R.: Schizophrenia and multiple sclerosis. Schiz. Bull., in press.

Stevens, J.R.: Psychiatric aspects of epilepsy. In Benson, F. (Ed.): Course Syllabus for Epilepsy and Behavior. American Academy of Neurology, April 1987, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02275-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Search for Virus in CSF and Postmortem Brain of Patients with Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Janice R. Stevens, M.D., Medical Officer, Neuropsychiatry Branch, IRP, NIMH

Dr. David Asher, NINCDS, Bethesda, Maryland; Dr. Joan Schwartz, NINCDS, Bethesda, Maryland; Dr. Peggy Swoveland, Dept. of Neurology, Univ. of Maryland; Dr. David Jacobowitz, NIMH, Bethesda, Maryland

COOPERATING UNITS *(if any)*

NINCDS
University of Maryland
NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Aging

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Based on evidence that an infectious agent or agents may play a role in the etiology of some schizophrenic illnesses, we are culturing fresh CSF from patients with schizophrenia and from control subjects on human neuroblastoma cells. Our previous studies have included immunocytochemical investigations for antigens to cytomegalovirus (CMV), herpes simplex virus (HSV), varicella virus, rubella and mumps. Although sporadic cases have shown positive results with immunocytochemical studies, these have been inconsistent and rare. We have also undertaken in situ hybridization probes for CMV, and cultivation of schizophrenic and control brain specimens on cultures of human and non-human neural tissue without success. Using special stains for glia we have evaluated the brains of guinea pigs and primates previously inoculated with schizophrenic and control brain tissue. During the past year we have investigated the effects of cerebrospinal fluid and sterile brain tissue from schizophrenic patients on the growth, peptide production and morphology of cultured human neuroblastoma cells. One line of human neuroblastoma cells (SHEP) has shown a consistent change in growth with loss of contact inhibition and piling up of cells to higher confluence commencing several months after incubation with schizophrenic CSF in 6 out of 7 cases, but in no control inoculated cells. Adenylcyclase production is increased in schizophrenic CSF treated cells and the effect on cell growth has been passaged with cell free media. Two dimensional electrophoresis gels demonstrate differences in protein in schizophrenic compared with control gels. Further studies in progress with these transformed cells include electron microscopic examination (Dr. Peggy Swoveland), studies for reverse transcriptase (negative), ELISA and immunocytochemical studies following application of control and schizophrenic sera to search for antibodies (negative so far).

GPO 914-918

Project Description:

Objectives: The purpose of this investigation is to search for evidence for an infectious or toxic agent as a significant etiologic factor in schizophrenia or a subgroup of schizophrenic patients. This work was stimulated by evidence from a number of sources including epidemiologic, immunologic, geographic and neuropathologic studies compatible with an infectious etiology in this disorder. Highlights of the evidence include increased immunoglobulin in the cerebrospinal fluid of schizophrenic patients to specific viral agents CMV, HSV, seasonal birth peaks of schizophrenic patients, uneven geographic distribution, abnormal response of lymphocytes to specific mitogens, toxic effects of urine, serum or cerebrospinal fluid of schizophrenic patients on animal behavior and tissue cultures, and gross and histologic neuropathologic changes in the brains of individuals with schizophrenia. Our previous attempts to identify antigens or viral genome from schizophrenic brains or passage of this disorder to animals or through cell cultures have generally been negative. We have now, however, just enough positive results to require extension, replication and introduction of more sensitive methods for identification of our transmissable factor. We are in an unusually favorable position to make such investigations.

Immunocytochemical studies with frozen and fixed schizophrenic brain specimens are being continued in collaboration with Dr. Maciej Poltorak. The present work is with schizophrenic and control fixed specimens with the antibody raised against phosphorylated neurofilaments considered to be a specific finding in soma of neurons in Alzehimer's disease. Many of the schizophrenic specimens are showing similar staining.

Significance to Mental Health Research: Schizophrenia is one of the most disabling neuropsychiatric problems in the United States, and indeed in the world. Schizophrenia affects approximately one percent of the U.S. population. It is young people who are most affected and 20-25 percent of those affected are severely and permanently handicapped despite use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required. The work is difficult and immediate rewards are few. Because of circumstantial evidence for infection as a significant cause of some schizophrenias, we are focusing our efforts on the search for an infectious agent. We are fully aware that this is "long-shot" research with no immediate promise of answers. Our success in obtaining cell transformation in 6 out of 7 schizophrenic cases studied and in no controls is very encouraging.

Proposed Course of Project: Our immediate plans for this project are to continue with the culture of CSF from schizophrenic and control patients and to investigate further the nature of the cell transformation seen to date. We also plan to continue immunocytochemical studies using specific antibodies against abnormal cytoskeletal features characteristic of Alzehiemer's disease and to apply them to schizophrenic material.

Publications:

Stevens, J.: The search for an anatomic basis of schizophrenia. In Mueller, J. and Yingling (Eds.): Perspectives in the New Neuropsychiatry. New York, Karger, in press.

Stevens, J.: Small heads and schizophrenia. Arch. Gen. Psychiat., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02277-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regional Cerebral Blood Flow in Neuropsychiatric Patients and in Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel Weinberger, M.D., Chief, Section on Clinical Neuropsychiatry, NPB, NIMH;
Karen Faith Berman, M.D., Staff Psychiatrist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02278-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Brain Imaging in Schizophrenic Patients and Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel R. Weinberger, M.D., Chief, Sec. Clin. Neuropsychiatry, NPB, IRP, NIMH

Dr. George Jaskiw, Vis. Assoc., Sec. Clin. Neuropsychiatry, NPB, IRP, NIMH; Dr. Barbara Illowsky, Med. Staff Fellow, Sec. Clin. Brain Studies, NPB, IRP, NIMH; Dr. Dilip V. Jeste, Med. Officer, NPB, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, NPB, IRP, NIMH; Dr. Allen Doran, Clin. Res. Assoc., Clin. Neurosci. Br., NIMH; Dr. Carl Feinstein, Asst. Prof. Psychiatry, George Washington Univ., Dr. David Pickar, Chief, Sec. Clinical Studies, Clin. Neurosci. Br., NIMH

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH
George Washington University

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02280-03 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brain Tissue Transplantation in Primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard Jed Wyatt, M.D., Chief, Neuropsychiatry Branch, IRP, NIMH

Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH; Dr. Richard Nakamura, LPP, IRP, NIMH, Dr. Donald Price, Johns Hopkins Hospital; Dr. Cheryl Kitt, Johns Hopkins Hospital

COOPERATING UNITS *(if any)*

LPP, NIMH
Johns Hopkins Hospital

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To advance the work already performed in our laboratory with rats, adrenal medulla was grafted to the denervated caudate of the rhesus monkey in our continuing research on brain tissue transplantation. Success for graft survival has been good in the last year. An instrument (the brain grafter) which facilitates grafting was developed.

GPO 914-918

Project Description:

Objectives: The objective of this program is to transfer knowledge gained from brain tissue grafts in the rodent into primates and eventually into humans.

Methods Employed: These studies involve surgical, histological and histochemical procedures in primates.

Major Findings: Considerable success has been achieved in grafting embryonic substantia nigra in young adult adrenal medulla in rats to decrease rotational behavior produced by unilateral lesions of the substantia nigra.

Prior to introducing brain grafts in humans, it is important to establish procedures in animals intermediate between the rat and man. For example, it is crucial to know if grafts survive and if so, how well in nonhuman primates. Our first series of rhesus macaque (Macaca mulatta) animals using both adrenal medulla and rhesus embryonic substantia nigra was, for the most part, unsuccessful. With one exception, grafts were not found.

A second series was slightly more positive; at least some tissue survived transplantation. Eight mature adult male rhesus macaque animals received a unilateral neurotoxic lesion of the region of the substantia nigra (including A10). At least two months later, each animal received a unilateral implant of either fetal rhesus substantia nigra tissue or tissue from its own adrenal medulla, and at least two months after implantation each animal was killed for catecholamine fluorescence histochemistry. The first two animals (A1 and A2) received fetal substantia nigra. The remaining animals (A3 through A7) received host adrenal medulla tissue.

For the embryonic substantia nigra implants, a rhesus monkey embyro (59 and 71 day) was surgically removed ex utero and placed in lactated Ringer's solution. The brain was dissected to obtain substantia nigra tissue in a manner analogous to that for embryonic rat brain. The region of the midbrain that includes substantia nigra tissue was divided into approximately 0.25 mm^3 pieces. This tissue was drawn into a 22-gauge needle with an average of six pieces of tissue per injection in a volume of approximately 10 to 20 μl of Ringer's solution.

After removing bone and dura, the injection needle was lowered into the head of the caudate nucleus with stereotaxic coordinates corrected by x-ray determination of skeletal landmarks. The needle was lowered until it was within the caudate. The tissue was then injected and after three minutes the needle was withdrawn.

For the six remaining animals (A3 through A8), the left adrenal was taken through a posterolateral retroperitoneal approach. A single longitudinal incision was made through the adrenal capsule and cortex under a dissecting microscope. The adrenal cortex was peeled off and cortical fragments trimmed away. The adrenal medulla was divided into pieces of 0.25 mm^3. The tissue in Ringer's solution was drawn into a 22-gauge needle with between 5 and 10 pieces of tissue in a volume of approximately 10 to 20 μl per injection.

In animal A3, stereotaxic placement of the adrenal graft was used as described above for the two animals implanted with fetal substantia nigra. A total of five injections were made into the caudate of animal A3. In the five remaining animals (A4 to A8), to provide more secure anatomic placement of grafts, the

caudate was directly observed. With the aid of a surgical microscope, a window was cut through the body of the corpus callosum exposing the left lateral ventricle and caudate. A 22-gauge needle was inserted into the body of the caudate to inject the tissue.

Surviving graft tissue could be identified by the presence of specific catecholamine histofluorescence in the cell bodies of the implants. Neither animal implanted with fetal substantia nigra had any evidence of surviving catecholamine-containing graft tissue. In contrast, the six animals implanted with host adrenal medulla had at least some surviving tissue in the parenchyma of the denervated caudate nucleus. The graft tissue itself appeared relatively healthy, although accumulation of macrophages was noted adjacent to or surrounding some of the graft sites. The only damage to host caudate associated with the implantation procedure was scar formation along the needle track.

Most graft sites were deep within the body of the caudate nucleus along the implant tract. Additionally, there were two graft sites that were on the edge of the caudate nucleus. At least some parts of most grafts appeared fused with the brain parenchyma, but there was no evidence of caudate reinnervation. All graft sites demonstrated some diffusion of the catecholamine. Most fluorescent cells retained the typical rounded appearance of adrenal chromaffin cells. A minority of cells developed polygonal shapes, and a few cells appeared to develop nerve-like fiber processes, though these remained within the graft itself. A third series of animals has been more successful but results are preliminary.

In addition to grafting tissue directly into the striatum of monkeys, tissue has been grafted into the frontal cortex. The advantage of grafting into the frontal cortex is that the surgery is considerably simpler than grafts into the striatum, and allows for developing surgical procedures which do not require lesioning animals and use of complex stereotaxic placement of grafts. In addition, in some cases animals do not need to be sacrificed to determine results. In this series of six animals over 10,000 adrenal chromaffin cells have survived.

During the last year an instrument has been built which allows us to carefully insert the grafts into the striatum using a stereotaxic instrument. This has increased graft survival in monkeys and we are applying for a patent.

The main grafter consists of a series of cannula designed to minimize tissue trauma and allow easy, precise placement within the brain. The principle involves the insertion of tissue housed in a protected enclosure; when the enclosure is withdrawn the graft is left in place without additional pressure. The device consists of an outer guide cannula and two sets of inserts. The first insert is an occluder used for initial penetration, only. After penetration, the occluder is removed. The second insert consists of an inner cannula fitted with a stylet. The amount of space the tissue occupies and therefore the size of the tissue is determined by the spacing of the stylet in the cannula. This spacing is controlled by a holder. The stylet is fixed to the holder; the cannula can be kept in a fixed position or allowed to slide on the stylet. Tissue is inserted into the tip of the inner cannula with the stylet fixed. The inner cannula is lowered and the tissue left in the brain by lifting the inner cannula while the position of its stylet is fixed.

Stereotaxic Instrument: For use in Macaca mulatta, the instrument is designed to fit into a modified Kopf stereotaxic instrument (Model 1404, David Kopf

Instruments, Tujunga, Calif.), although modifications can be made for use in other stereotaxic instruments. The stereotaxic frame assembly is equipped with two carriers (Kopf model 1460), A and B; one carrier on each frame bar.

Brain Grafter Construction: The brain grafter is made from stainless steel or another rigid, sterilizable material. It consists of two cannula assemblies, A and B. Cannula assembly A consists of an outside guide cannula and a stylet for making initial penetration into the brain. Cannula A is affixed to a 10 mm long cuff or holder that fits over one end of the cannula. The tubing of cannula A extends 84 mm beyond its 10 mm cuff. The tubing has a .228 mm wall with an outer diameter of 1.65 mm and an inner diameter of 1.193 mm. Stylet A extends 95 mm beyond a holding knob. The knob has been trimmed on one side so that it can easily pass up and down as the two carriers vertically move past one another. Stylet A is brought to a point extending 1 mm beyond outside cannula A. A bevel on stylet A is tappered to be continuous with a similar bevel outside cannula A allowing for smooth penetration into the brain.

Cannula assembly B consists of an outer cannula which extends 94 mm beyond its 10 mm cuff. Its inside diameter is .685 mm, the outside diameter is 1.066 mm and it has a wall thickness of .177 mm. Its tip is beveled to give a cutting edge for punching tissue. Stylet B with a diameter of .558 mm extends 105 mm beyond its 10 mm long cuff.

Cannula assembly B fits into a holder assembly H. Holder assembly H consists of a hollow tube with a 1 mm wide viewing slot cut 1.5 cm lengthwise. On the edge of the viewing slot are one mm marks for determining the distance between the cuff of stylet B and the cuff of cannula B. A set screw in H holds stylet B in a permanent mount in the barrel of the cannula assembly holder H. Thumb screw H on the cannula assembly holder H maintains cannula B in a fixed position in relation to the stylet B.

Use of Brain Grafter: During surgery, cannula assembly A with stylet A is stereotaxically lowered through a burr hole to a position where the graft is to rest. Stylet A, is removed. Cannula assembly B and holder assembly H are used to keep the stylet and the cannula at a fixed distance to allow the donor tissue to be punched and taken into the cannula. Using the millimeter markings on the view slot of holder H to determine the amount of tissue to be grafted, thumb screw H is tightened around cannula B. (For example, when the amount of tissue to fill the cannula is determined to be 2 mm, the two cuffs of the cannula assembly are placed 2 mm apart as determined by the view slot and markings.) Thumb screw H is tightened and the cannula assembly is used to punch the tissue to be grafted. Following filling of the cannula assembly B with the punched material, cannula assembly holder H is inserted into cannula assembly A. Stereotaxic carrier B is lowered onto assembly B and locked in by tightening its carrier screw. Thumb screw H is loosened, and cannula assembly A raised until contact is made with cuff B. As cannula B is raised, the tissue is dropped from the cannula and deposited in its proper place. Multiple injections can be made into the same track by simply raising cannula assembly A to the appropriate height and repeating the procedure.

Experience has taught us that cannula B's cross sectional sizes are the smallest that we can reliably use to punch adrenal medulla from the monkey. Dimensions of the other cannula and stylets are determined by cannula B. Preliminary data indicate that the device may be superior to other techniques for transplantation

of adrenal medulla into the primate striatum. In a number of sites tens of thousands of cells have survived; in other sites only a few cells survive. While the number of surviving cells is inconsistent, the grafter gives better maximum survival of adrenal chromafin cells than other techniques we have used in monkeys. The yield (survival of cells) using this device is also superior to what we have found in the parenchyma of the rat brain where about 200 chromafin cells per animal survive permanently when simply injected with a needle into the striatum.

Significance to Mental Health Research: These studies may lead to the development of tissue transplantation as a therapeutic procedure for degenerative diseases and destructive lesions of the brain in the clinic. Also, they may lead to increased knowledge about development and regeneration in the brain in general. Since there is considerable evidence that some schizophrenic patients have altered brain structure, perhaps through degeneration, and degeneration is clearly involved in diseases such as Alzheimer's disease, learning more about brain plasticity is of primary importance in understanding these illnesses.

Proposed Course of Project: Brain grafting should be seen as both a potential treatment for disorders such as Parkinson's disease as well as a potential for understanding plasticity in general. The course of this project should continue until such time as there is sufficient justification for bringing these techniques on a widespread basis into the clinic. At that time further refinements and developments will probably be needed in order to maximize potential benefits to patients. Because work with primates is inherently slow, progress will also be slow, but nevertheless, there does appear to be incremental enhancements of our ability to graft tissue in primates over the last few years. We would expect this progress to continue over the next several years.

Publications:

Wyatt, R.J., and DeRenzo, E.G.: Deinstitutionalization: "For every complicated problem there is a simple solution and that solution fails" (H.L. Mencken). Stanford University, in press.

Liebowitz, M.R., Quitkin, F.M., Stewart, J.W., McGrath, P.J., Harrison, W., Karoum, F., Wyatt, R.J., Levitt, M., Rabkin, J., and Klein, D.F.: Efficacy of L-deprenyl in atypical depression: A preliminary report. Monograph, Chinoin Pharmaceutical Incorporated. Budapest, Hungary, in press.

Potkin, S.G., Bell, K.M., and Wyatt, R.J.: The relationship between monoamine enzymes and schizophrenia. In Handbook of Studies in Schizophrenia, III Psychobiology, in press.

Wyatt, R.J., Kirch, D., and DeLisi, L.: Biochemical studies of schizophrenia. In Kaplan, H. and Sadock, B. (Eds.): The Comprehensive Textbook of Psychiatry, 5th Edition. Baltimore, Williams and Wilkins, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02281-03 NPB

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Neural Tissue Microchip Interface

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor) (Name, title, laboratory, and institute affiliation)*
A. Paul Oliver, Physiologist, Neuropsychiatry Branch, IRP, NIMH

Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH; Dr. Myles Jaffe, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Marty C. Peckerar, Naval Research Laboratory, Microelectronics Processing Facility, Washington, D.C.

COOPERATING UNITS *(if any)*

Naval Research Laboratory, Microelectronics Processing Facility, Washington, D.C.

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of a neural prosthesis requires the fabrication of a solid state device capable of recording information from neural tissue and stimulating it in a timely manner. We began this process by designing a simpler system to test and monitor cultured neural tissue. To date we have identified problems such as suitable insulation for the device and some problems with corrosion in a critical part of the system. In addition we have improved the tissue culture system in an attempt to make the tests as realistic as possible. We have also worked with cultured retinal tissue in the system so that two way communication could be achieved.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: The development of practical neural prosthetic devices offers a potential method for replacement of damaged or destroyed neuronal tissue. In the early design stages of this project many difficult technical problems must be solved. To do this a simpler system for communicating with animal nervous systems is being developed. One device is part of a tissue culture system for long term recording of cultured nerve cells. A second device for in vivo application is being fabricated. Both will be integrated with a computer for signal analysis.

Methods Employed: Both devices are fabricated with photolitographic techniques at the Naval Research Laboratory. The computer interface electronics have been designed and built by the NIMH Intramural Program Technical Development Service. The tissue culture chip will record a minimum of 30 channels with one or more nerve cells per channel, a Dec Computer will store and analyse the information. The in vivo system has 40 channels and is designed for insertion into brain structures such as cerebral cortex. It will be used for simultaneous recording of nerve cells in a cross-section of a given brain structure.

Major Past Findings: The recording system is workable, but it needs more insulation to prevent signal attenuation. The system is vulnerable to corrosion when used over long periods of time. It is difficult to coat the probe so that cultured tissue will stick to the probe.

New Findings: Retinal tissue can be cultured and kept in vitro for considerable periods of time. However, we have not yet demonstrated light responses from this tissue. We have developed a new technique using a substance called Matrigel, a commercial product, which allows retinal growth approximating that seen with in vivo retinal transplants. We expect that this will result in a workable system.

Significance to Mental Health Research: The development of prostheses and the method of grafting tissue to the brain both offer potential methods for restoring function to damaged tissue. The work done on prosthesis design, and the tissue culture studies will contribute toward the attainment of these goals.

Proposed Course of Project: Development and testing of communication devices will continue in this laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02282-03 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurovirology and Neuroimmunology of Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Darrell G. Kirch, M.D., Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Rita Anand, Special Expert, NPB, IRP, NIMH; Dr. Anita Feenstra, Visiting Associate, NPB, IRP, NIMH; Dr. Nicholas M. Papadopoulos, Clinical Chemistry Service, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

Clinical Chemistry Service, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project on neurovirology and neuroimmunology has continued its efforts to provide evidence that the pathogenesis of schizophrenia involves either an infectious process by a viral agent and/or an autoimmune reaction involving central nervous system tissue autoantibodies.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description:

Objectives: In spite of a vast number of studies showing both structural and functional abnormalities in the central nervous system in schizophrenia, the etiology or etiologies of this disorder are unclear. This project is intended to make use of newly developed techniques in molecular biology, virology, and immunology, to study patients with schizophrenia searching for a possible viral and/or autoimmune cause.

Methods Employed: Cerebrospinal fluid (CSF) samples are analyzed using rate nephelometry in order to measure both albumin and immunoglobulin G (IgG). If these components of CSF and serum are measured, one can estimate endogenous IgG production in the central nervous system.

Lymphocytes are being harvested from patients and established in tissue culture in Dr. Feenstra's laboratory. The methods used in her laboratory to study these tissue cultures for evidence of a retrovirus infection are described under a separate project heading. In addition, Dr. Anand, a virologist, has joined the Neuropsychiatry Branch and will be establishing a laboratory that will study the biological effects of retroviral infections in the central nervous system. This will make use of tissue culture techniques using both peripheral lymphocytes and brain tissue. Attempts will also be made to search for evidence of a viral infection using DNA and RNA hybridization methods.

Major Past Findings: In past studies, a subset of patients with chronic schizophrenia were found to have increased central nervous system IgG production. In the case of one patient there was evidence of oligoclonal banding when electrophoresis of CSF was performed.

New Findings: Initial attempts to study lymphocyte cultures for evidence of a retroviral infection have been negative.

Significance to Mental Health Research: Although studies in this area have yet to identify firm evidence of viral infection in schizophrenia, the goals of the project remain important. If a viral infection and/or autoimmune process are found to be involved in even a subset of schizophrenia patients, this would be an important advance in understanding the etiology of this disorder. Moreover, the discovery of more effective treatments (or possibly the prevention of) schizophrenia is dependent upon a better understanding of the cause of the disorder.

Proposed Course of Project: The current emphasis in the project will be on using a variety of tissue culture methods and DNA/RNA hybridization methods to search for viral factors in samples from patients with schizophrenia. There will also be ongoing emphasis on studies of CSF proteins to look for indirect evidence of viral infection and/or autoimmune response.

Publications:

Kirch, D., and Weinberger, D.: Post-mortem neuropathology. In Nasrallah, H., and Weinberger, D. (Eds.): Handbook of Schizophrenia, Vol. 1: The Neurology of Schizophrenia. Amsterdam, Elsevier, 1986, pp. 325-348.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02309-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Brief Psychiatric Rating Scale as a Ward Daily Rating System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Llewellyn B. Bigelow, M.D., Associate Clinical Director for the William A. White Division, Saint Elizabeths Hospital

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Office of the Clinical Director of DIRP, NIMH.

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02310-02 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Treatment of Migraine with Anionic Polyelectrolytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Llewellyn B. Bigelow, M.D., Associate Clinical Director for the William A. White Division, Saint Elizabeths Hospital

Dr. Ernst Thonnard, General Medical Officer, Saint Elizabeths Hospital

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
This project has been transferred to the Office of the Clinical Director of DIRP, NIMH.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02311-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Ontogeny of Preprocholecystokinin, Proenkephalin and Tyrosine Hydroxylase in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Anne-Marie Duchemin, M.D., Visiting Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH; Dr. Michael Iadarola, Neurobiology-Anesthesiology Branch, NIDR, NIH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

Neurobiology-Anesthesiology Branch, NIDR, NIH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

Brain peptides are neurotransmitters or neuromodulators in the adult brain. In the embryonic brain, they may play a role in the organization and development of neurons and synaptogenesis. We studied the ontogeny of cholecystokinin (CCK) and enkephalin in the rat brain from day 12 prenatal to adulthood by measuring the peptides and the mRNA levels in parallel in the same tissues. CCK-immunoreactive peptides (CCK-IR) and enkephalin immunoreactive peptides (enkephalin-IR) were measured by using a specific radioimmunoassay. Messenger RNA levels were measured by blot hybridization of poly(A)$^+$ RNA with the corresponding cDNA probes. We also studied the ontogeny of tyrosine hydroxylase (TH), the limiting enzyme in the synthesis of noradrenaline and dopamine. The monoamines, their metabolites and the mRNA levels were measured comparatively in substantia nigra and locus ceruleus of the rat during development.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: The ontogeny of brain peptides has been studied by radioimmunoassay and by histochemical analysis. In this report, we have examined the expression of the CCK, enkephalin and tyrosine hydroxylase genes by measuring in parallel mRNA and the translational product in the rat brain during embryonic and post-natal development.

Methods Employed: Quantification of preproCCK, proenkephalin and TH mRNAs was made by Northern blot analysis using the corresponding cDNA hybridization probes. RNA from whole brains and from several brain regions was prepared by the guanidium thiocyanate cesium chloride method. Poly $(A)^+$-RNA was purified by affinity chromatography on oligo dT cellulose.

RNA was electrophoresed on formaldehyde-agarose gel and transferred to nitro-cellulose paper. RNA blots were hybridized with the ^{32}P-nick translated cDNA probes. Quantitative analysis of the autoradiographs was performed by densitometric scanning.

In the case of CCK and enkephalin studies, peptide levels in the same age brains were measured by radioimmunoassay. For the TH study, noradrenaline, dopamine, HVA, DOPA and MHPG were measured by mass spectrometry.

Major Findings:

Developmental regulation of CCK gene: Brain preproCCK mRNA was detectable at embryonic day 14. It increased progressively to reach maximum levels two weeks after birth and tended to decrease in adult rats.

CCK-IR was undetectable at embryonic day 14. Detectable levels were measured by embryonic day 20, but marked development was seen only after birth with a rapid increase between post-natal days 7 and 21, the CCK-IR content per brain increasing 10 fold during this period. By three weeks, the content reached approximately 90% of the adult level.

Development regulation of enkephalin gene: Brain proenkephalin mRNA appeared much earlier than CCK and was already detectable at embryonic day 12. It was maximal at day 15.

Enkephalin peptides were present as soon as day 12 prenatal. The peptides showed a marked increase just after birth and then declined to reach adult level at the second week post-natal. Surprisingly, the peak of enkephalin-like peptides occurring during the first week post-natal was not correlated with an increase of proenkephalin mRNA. This suggests that regulatory mechanisms occurred not at the synthesis level but at the post-translational processing or peptide degradation levels.

Comparative study of the expression of enkephalin and CCK genes showed a large discrepancy in the time-course of the developmental expression of the two genes. The enkephalin gene expressed very early in the embryo and may be involved in embryonic development of the brain.

The CCK gene expressed during synaptogenesis could be involved in post-natal oraganization of the brain, possibly as a trophic substance for synaptogenesis or afferent innervation.

Developmental regulation of the tyrosine hydroxylase gene: Mass spectrometry measurements of catecholamines and their metabolites in the brain showed that noradrenaline appeared before dopamine. These two monoamines have the same limiting enzyme - tyrosine hydroxylase - involved in their synthesis process. Thus, we were interested in comparing the expression of the tyrosine hydroxylase gene in two different brain regions, one which is rich in noradrenaline - the locus ceruleus, and the other rich in dopamine - the substantia nigra.

Total RNAs prepared from locus ceruleus and substantia nigra from embryonic and post-natal rats were hybridized with the ^{32}P-tyrosine hydroxylase cDNA probe. Analysis of the data is in progress.

Significance to Mental Health Research: The neurotransmitters we are studying (CCK, enkephalin, DA, NE) could be involved in the pathogeny of mental disorders. Comparative studies of the development of these neurotransmitters in the central nervous system would allow a better understanding of the factors regulating the expression of the corresponding genes.

Proposed Course of Project: The current data obtained will be analyzed and processed for publication. The utilization of the cDNA probes will be extended to the study of the distribution of the corresponding mRNAs in discrete regions of the rat brain and in normal and schizophrenic human brains.

Publications:

Duchemin, A.M., Quach, T.T., Iadarola, M.J., Deschenes, R.J., Schwartz, J.P., and Wyatt, R.J.: Expression of the cholecystokinin gene in rat brain during development. Dev. Neurosci. 9: 61-67, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02312-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Neurotrophic Activity in Cerebrospinal Fluid of Schizophrenic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Anne-Marie Duchemin, M.D., Visiting Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Thanh Tam Quach, Visiting Associate, Neuropsychiatry Branch, IRP, NIMH; Dr. Charles Kaufmann, Staff Psychiatrist, Neuropsychiatry Branch, IRP, NIMH; Dr. Daniel Weinberger, Chief, Clinical Brain Disorders Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

We used cultures of sympathetic neurons from chicken embryo ganglia as an assay for neurotrophic activity. Introduction of radioactive tracers has been developed to facilitate quantification of neurotrophic activity. We looked for neurotrophic activity in CSF of normal volunteers, neurologic and psychiatric patients. There was no evidence of neurotrophic activity in most of the CSF tested. However, CSFs of some schizophrenics were found able to support neuronal survival in culture. This activity was independent of neuroleptic treatment but was positively correlated with ventricular enlargement of the brain.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: A culture of neurons provides convenient assays for trophic factor activity. The survival of the neurons in response to an added factor can be quantified and used as a measure of the amount of trophic activity present.

Methods Employed: Sympathetic ganglia form 12-day-old chicken embryos were dissected, and, after trypsin dissociation, the suspended cells were plated in the presence of CSF. After 24 hours culture, the plates were analyzed for neuron survival. Neuron survival was estimated by counting neurons under a phase microscope after fixation and coloration. We developed other methods to quantify neuronal survival. The addition of radioactive tracers to the cell culture and the measure of their incorporation in the cells gave an index of the cell survival. We found a good correlation between incorporation of ^{35}S-methionine or ^{3}H-uridine and the count of surviving neurons and used these tracers in our assays of neurotrophic activity.

Major Findings: Neurotrophic activity has been shown to appear in the rat brain after lesion. The activity is time-dependent with a maximal activity seven days after lesion and a return to normal within three weeks. The activity has been correlated with the increase of graft survival in the wound cavity. Such an activity has also been shown to exist in the human CSF of patients with head trauma. We looked for the presence of neurotrophic activity in the CSF of neurologic and psychiatric patients.

We found that CSF from neurologic or non-schizophrenic patients and from normal controls does not allow the neurons to survive in culture. But CSF from some schizophrenic patients was found to contain a neurotrophic activity for sympathetic neurons. Patients with CSF containing the higher neurotrophic activity appeared to have enlarged ventricles as measured on computed tomography. Neuroleptic treatment does not seem to be responsible for the neurotrophic activity. Characterization of this neurotrophic activity showed that it is destroyed by heat. Dilution curves of CSF from controls and from schizophrenic patients showed no evidence of neurotoxicity in controls and that the neurotrophic activity in schizophrenic CSF is dose-related. Neurotrophic activity of psychotropic drugs has also been investigated.

Significance to Mental Health Research: Neurotrophic factors could be related to brain injury or brain atrophy. The study of their variations in man under different pathological processes could be of interest for the understanding of the pathogeny of some degenerative disorders of the brain.

Proposed Course of Project: The comparison of the characteristics of these factors from human CSF with the factors obtained from injured rat brains will be pursued. Neurotrophic activity of CSF will be tested on other cell lines, particularly on neuroblastoma cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02313-02 NPB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Retroviral Activity in Lymphocytes of Patients with Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Anita Feenstra, Ph.D., Visiting Associate, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell G. Kirch, Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

The evidence that schizophrenia may involve infection by a virus (or viruses) has been indirect. This evidence includes the phenomenology of schizophrenia (insofar as it may be mimicked by some viral encephalitides), epidemiological factors (including a predominance among patients with late winter/early spring births, a north-south gradient, and occasional clustering of cases), and indirect laboratory evidence (gliosis in some neuropathological studies, spinal fluid protein abnormalities, and abnormalities in cell-mediated immunity).

The discovery of the human retroviruses, HTLVI, HTLVII and HIV, now also known to affect the CNS, together with the development of new techniques in human retrovirology, made it possible to investigate the role of this class of viruses in the etiology of schizophrenia.

Cultures of peripheral lymphocytes of patients with chronic schizophrenia were established and tested for the retrovirus-specific enzyme reverse transcriptase.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description:

Objectives: A viral infection has been proposed as a possible etiology of schizophrenia. The discovery of several human retroviruses involved in malignancy as the HTLV retrovirus family has renewed our interest in this hypothesis. We choose to culture the lymphocytes of patients with schizophrenia because these cells circulate through the body, are in contact with the brain and can be cultured up to a month without being transformed. As an initial screening, the lymphocyte cultures are tested for the retrovirus specific enzyme reverse transcriptase. If positive, the cells are stained with different available antibodies against human retroviruses. The positive cells are co-cultured with cells susceptible to viral infection to enrich for viral particles in an attempt to identify and possibly isolate a virus associated with schizophrenia.

Methods Employed: Lymphocytes are isolated from peripheral blood of patients with schizophrenia and matched controls. The cells are cultured after stimulation with PAH in the presence of T-cell growth factor. During a period of 30 days the culture is tested for reverse transcriptase activity.

New Findings: Short term tissue cultures of peripheral lymphocytes from 20 chronic schizophrenic patients and 10 normal subjects were established. The cells were grown in the presence of T-cell growth factor, and the culture supernatant was tested for the presence of reverse transcriptase. No T-cell associated with reverse transcriptase activity has been detected in our cultures from patients or normals under the present conditions.

Significance to Mental Health Research: The existence of neuronotrophic viruses and the study of their effect on brain function and development opens a new area of brain research. Their possible involvement in brain diseases might result in a better understanding and treatment of the disease.

Proposed Course of Project: Lymphocyte cultures of patients with schizophrenia will be established and stimulated in various ways to induce viral activity. Patients are being tested for several other neuronotropic viruses, as HIV and HBLV. Cocultures of patient lymphocytes and susceptible cells will be established in order to enrich for virus producing cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02314-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an Auditory Sort Test

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Neuropsychiatry Branch, IRP, NIMH

Dr. Daniel Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. Craig Karson, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Karen Berman, Staff Psychiatrist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02315-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hierarchy and Sensitivity in Putative Frontal Lobe Tasks

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Neuropsychiatry Branch, IRP, NIMH

Dr. Daniel Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH;
Dr. John Kelsoe, Section on Clinical Studies, Clinical Neuroscience Branch, NIMH

COOPERATING UNITS *(if any)*

Section on Clinical Studies, Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [x] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02316-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

"Teaching" the Wisconsin Card Sort to Schizophrenic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Neuropsychiatry Branch, IRP, NIMH

Dr. Daniel Weinberger, Chief, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. Karen Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Marvin Podd, O'Malley Division, Saint Elizabeths Hospital

COOPERATING UNITS *(if any)*

O'Malley Division, Saint Elizabeths Hospital

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.33	0.17	0.17

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02317-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Peripheral and Central Metabolism of D- and L-DOPA in Rats

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboretory, and institute affiliation)*

Farouk Karoum, Ph.D., Chemist, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Psychopharmacology

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.33	0.67

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The effects of administering deuterated L-DOPA on the formation and metabolism of peripheral and central non-deuterated catecholamines as described in the 1986 Annual Report is now completed. Our interest had shifted towards comparing central and peripheral metabolism of D-DOPA with that of L-DOPA.

We found that both D- and L-DOPA gave rise to DA by about the same efficiency. This unexpected finding led us to characterize these two amino acids biochemically as well as behaviorally. We also carried out additional experiments in order to explore the underlining mechanisms that are responsible for the formation of dopamine from D-DOPA.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Methods Employed: All biochemical analyses were performed by combined gas chromatographic mass spectrometric methods developed in this laboratory. In some studies rats with unilaterally lesioned substantia nigra with 6-hydroxydopamine were used.

Major Findings:

1. In the intact rat, intragastric administration of D-DOPA together with carbidopa (alpha methyl dopa hydrazine, a peripheral dopadecarboxylase inhibitor) increased striatal and hypothalamic dopamine concentrations to the same extent as a similar treatment with L-DOPA plus carbidopa.
2. In rats with unilateral 6-hydroxydopamine-induced lesions of their substantia nigra, both stereoisomers of DOPA produced significant increases in dopamine and its metabolites in the intact striata. Although dopamine concentrations in the lesioned striata did not change, a significant increase in dopamine metabolites was observed, indicating some extraneuronal formation of dopamine. These results suggest that D-DOPA can be converted to dopamine in the normal striatum as well as in the striatum devoid of dopamine nerve terminals.
3. D- and L-DOPA produced turning behavior in unilaterally lesioned rats with a similar efficacy. The onset of turning after D-DOPA was delayed compared with L-DOPA. Turning behavior elicited by these amino acids was attributed to stimulation of supersensitive dopamine receptors in the lesioned striata by the small amounts of extraneuronally formed dopamine.
4. Preliminary results suggest that D-DOPA is converted to dopamine via transamination and/or D-amino acid oxidation to 3,4-dihydroxyphenylpyruvic acid, which, upon further transamination, gives rise to L-DOPA and hence dopamine.
5. The relatively fast and slow onset of stimulation of DA receptors by L-DOPA and D-DOPA respectively suggests that the use of the racemic mixture of DOPA combined with a peripheral dopadecarboxylase inhibitor may prove useful in the treatment of parkinsonism.

Significance to Mental Health Research: The above study as well as that reported in 1985 provided us with a number of new pieces of information regarding the metabolic fate of ingested L-DOPA and the role played by dopadecarboxylase inhibitor in the overall therapeutic benefits of L-DOPA. This information may prove useful in the future design of L-DOPA treatment in parkinsonism. For example, the lack of effect of alphamethyldopa on central metabolism of L-DOPA as opposed to its potent effect on brain norepinephrine atests to the safe use of this compound during L-DOPA therapy. Furthermore, if some of the beneficial effects of combined carbidopa treatment with L-DOPA are due to the metabolism of carbidopa to alphamethyldopa, then the combination of both carbidopa and alpha-methyldopa with L-DOPA is expected to offer additional advantage over combined carbidopa and L-DOPA only.

The main finding in this study is that D-DOPA can increase brain dopamine as efficiently as will L-DOPA. It is therefore possible that the coadministration of D,L-DOPA with a peripheral dopadecarboxylase may prove more beneficial to Parkinsonian patients than will L-DOPA plus carbidopa. This is because the former will be expected to have a longer course of effect than the latter.

Proposed Course of Project: It is hoped that the knowledge acquired from this study will be applied to the design of new treatments in parkinsonism. To achieve this goal, these new treatments will have to be tested on monkeys pretreated with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). MPTP treated monkeys provide a useful animal model of parkinsonism. Therefore, the course of future research will include both biochemical and behavioral evaluations.

The effect of D-DOPA on monkeys rendered will be tested both biochemically and behaviorally. This study is hoped to be a prelude to testing the efficacy of D-DOPA in parkinsonism. More studies will also be carried out to characterize the effects of D- and L-DOPA on peripheral biogenic amines.

Publications:

Karoum, F., Freed, W.J., Chuang, L.-W., Cannon-Spoor, E., and Wyatt, R.J.: D-DOPA and L-DOPA similarly elevate brain dopamine and produce turning behavior in rats. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02318-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Retinoic Acids on Brain, Behavior, and Drug Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gregory M. Straw, M.D., Clinical Research Associate, Preclinical Neurosciences Section, NPB, IRP, NIMH

Dr. Darrell Kirch, Associate Clinical Associate, NPB, IRP, NIMH; Dr. William J. Freed, Chief, Preclinical Neurosciences Section, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	0.25	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the pathophysiology of retinoic acid action in vivo. Rats are the current model but expansion into mouse and guinea pig models is anticipated. The primary focus of the rat model is the assay of the pharmacokinetics of the interaction of 13-cis-retinoic acid with neuroleptics. Early results have shown statistically significant changes in the blood levels of haloperidol and one of its metabolites after the concurrent administration of 13-cis-retinoic acid to rats. Early results also suggest possible interactions with dopamine metabolism and opiate levels in the CNS.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: (1) To delineate the pharmacokinetics of the interaction of retinoic acids and neuroleptics in multiple animal species; (2) To define any direct effects of exogenous and/or endogenous retinoic acids on behavior in animal models. The animal models of greatest interest are those that have already shown some ability to detect changes in dopaminergic function or to detect changes in the threshold for seizures or kindling. Many of these models are sensitive to or require the use of neuroleptic or related drugs. Therefore, the first objective clearly is to define the nature of any pharmacokinetic interaction of the retinoic acids with the neuroleptics.

Methods Employed: Male Sprague-Dawley rats were used in the initial experiments. They received intraperitoneal 13-cis-retinoic acid and subcutaneous haloperidol in five doses over two and one half days. The concentration of haloperidol in the blood was then measured by high pressure liquid chromatography. Mice and rats are tested for locomotor activity habituation and for learning and memory.

New Findings: In the rat, 13-cis-retinoic acid increases the serum haloperidol concentration as well as the hydroxy-haloperidol concentration. Also, as the retinoic acid dose rises there is a significant drop in the ratio of hydroxy-haloperidol metabolite to haloperidol. Mice show a direct neuroleptic-like effect of isotretinoin in locomotor habituation testing.

Significance to Mental Health Research: Retinoic acids are endogenous compounds that are known to modulate diverse cellular processes. They are also used clinically in treating skin ailments and are the center of focus for some cancer research. It is important to know the nature of their interactions with drugs that are given concurrently to patients with psychiatric diagnoses. However, it may be even more important to know if they have direct effects in these same diagnostic entities.

Proposed Course of Project: (1) Proceed with the careful elucidation of the pharmacokinetics of the rat, mouse and guinea pig models using haloperidol as the neuroleptic model. (2) Proceed in parallel with simple animal behavioral models to examine any direct effects, such as increased or decreased activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02320-02 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Magnetic Resonance Imaging (MRI) Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel R. Weinberger, M.D., Chief. Section on Clinical Neuropsychiatry, NPB, IRP, NIMH

Dr. Jean L. Cadet, Medical Staff Fellow, Section on Clinical Neuropsychiatry, NPB, IRP, NIMH; Dr. John Kelsoe, Medical Staff Fellow, Clinical Neuroscience Branch, NIMH; Dr. David Pickar, Chief, Section on Clinical Studies, CNB, IRP, NIMH

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH; Section on Clinical Studies, CNB

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Section on Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	0.67

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This project has been transferred to the Clinical Brain Disorders Branch.

PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02373-01 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effects of Cocaine on Central and Peripheral Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ralph W. Fawcett, M.D.,Ph.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Farouk Karoum, Chemist, Neuropsychiatry Branch, IRP, NIMH; Dr. Richard Jed Wyatt, Chief, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chronic administration of cocaine, 10 mg/kg twice daily for three weeks significantly reduced total body turnover of dopamine, but not that of norepinephrine. In another study, this effect was found to persist at six weeks after the termination of a three week cocaine treatment. Thus, it appears that cocaine has a long lasting and specific effect on peripheral dopaminergic systems.

In the brain, chronic cocaine appears to have most of its effects on dopamine nerve terminals in the frontal cortex. In other brain regions such as the hypothalamus, hippocampus, caudate nucleus, and the septum, appeared not to be affected.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: The object of this investigation is to test the suitability of rats chronically treated with cocaine as an animal model of cocaine.

Methods Employed: All biochemical analyses were performed by combined gas chromatographic mass spectrometric methods developed in this lab.

Major Findings:

1. Three weeks of chronic cocaine treatment significantly reduced total body dopamine turnover without any effect on norepinephrine turnover.

2. The above changes in dopamine and its metabolites was found to persist in rats killed six weeks after the termination of chronic cocaine treatment.

Significance to Mental Health: Cocaine abuse has become a major problem in this country. In addition to the euphoria and psychotic effects commonly discussed, there is evidence that chronic use of the drug may cause vegetative symptoms similar to the negative symptoms of schizophrenia. Therapeutic options will, of course, develop as the biochemistry at the neuronal level becomes known.

Proposed Course of Project: The effect of cocaine and dopamine in the periphery of the brain will be correlated to a number of pharmacological and behavioral parameters. These studies are hoped to assist us in our assessment of rats chronically treated with cocaine as an animal model of schizophrenia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02374-01 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Trial of Isotretinoin in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Darrell G. Kirch, Senior Staff Fellow, NPB, IRP, NIMH; Dr. Myles Jaffe, Senior Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Open clinical trials of isotretinoin in schizophrenic patients on and off of haloperidol have suggested a possibly beneficial synergism between haloperidol and isotretinoin in the treatment of psychotic illness. This has been supported by the results of animal studies of the direct and synergistic (with haloperidol) effects of isotretinoin on animal behavior. Therefore, this project has been initiated to evaluate these potential effects in a placebo controlled, double blind, cross over study. Also, other possible effects including changes in visual and auditory sensory processes will be monitored electrophysiologically.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: Examine the clinical effects of isotretinoin in a schizophrenic population.

Methods: The patients will have been diagnosed according to DSMIII criteria, and will be followed with serial psychiatric and nursing ratings and medical and psychological testing. Visual and auditory evoked potentials will be tested, as well as neuroleptic drug levels.

Major Past Findings: Decreased haloperidol blood levels and decreased psychiatric ratings for psychopathology have been seen in open clinical trials.

New Findings: Animal studies suggest isotretinoin may have direct effects of possible benefit separate from interaction with haloperidol.

Significance to Mental Health Research: The retinoids may comprise a group of compounds with novel ranges of benefit and side effects that could provide alternate treatments for psychoses.

Proposed Course: Clinical trials are to be completed by June 1989.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02375-01 NPB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Seasonality of Birth and Hospitalization for Schizophrenic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Gregory M. Straw, M.D., Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

Dr. Guido Zami, Ph.D., Information Systems, Saint Elizabeths Hospital; Dr. James Lohr, Medical Staff Fellow, Neuropsychiatry Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Preclinical Neurosciences Section

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many authors have reported seasonal variations in the evident birth rate and admission rate for schizophrenic patients. This project uses computerized hospital records and weather data to examine these possible "seasonal" effects.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: Mathematical and statistical description of any evidence of interaction between cyclic environmental changes and rates of birth or admission of schizophrenic patients.

Methods: Computerized data bases of hospital records and weather data and astronomical cycles will be compared.

Major Past Findings: Other authors report some evidence of links between season of birth and admission rates for schizophrenic patients.

New Findings: Our analysis is incomplete at this time.

Significance to Mental Health Research: The nature of environmental effects on birth and admission rates may assist in formulating testable theories for underlying pathologic mechanisms.

Proposed Course: Analysis to be completed by June 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01532-10 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Catecholamine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

De-Maw Chuang Group Chief LPP-NIMH

COOPERATING UNITS *(if any)*

Carmine Coscia, St. Louis University Medical School
Graig Malbon, State University of New York at Stony Brook
Hannu Alho, Fidia Georgetown Neuroscience Institute

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Receptor Pharmacology Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL	OTHER:
0.4	0.4	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The number of B-adrenergic receptor (BAR) fluctuates with changes in the neuronal activity in vivo. Two extremeties of this receptor plasticity are the supersensitivity and subsensitivity; the latter often involves the loss of receptor site, termed down-regulation. We have used a model system of frog erythrocyte to study the molecular mechanisms of B-receptor down-regulation induced by isoproterenol stimulation. Our previous studies have provided the first evidence that agonist-induced down regulation of BAR is associated with internalization of BAR sites in frog erythrocytes. This receptor internalization is causative for the desensitization of adenylate cyclase to BAR stimulation. Internalized BAR sites are sequestered in endocytotic vesicles with molecular weight more than 20×10^6 daltons and are recycled to the plasma membrane during receptor resensitization. Internalized BAR can be labeled by a lipophilic but not by a hydrophilic receptor ligand. Moreover, mechanisms of coated pit and coated vesicle may be involved in this receptor internalization and down-regulation. In the present study we have succeeded in staining BAR in frog erythrocytes using BAR specific antibody and detected important differences in the receptor staining pattern during receptor desensitization and down-regulation. Specifically, preceding the internalization of BAR, the staining of BAR on the plasma membrane by the BAR antibody was markedly enhanced. Internalization was associated with an increased labeling of a population of BAR which were unmasked by permeabilization of the cells with the detergent saponin. Currently we are attempting to elucidate the details of molecular events at the electron microscopic level using this morphological approach. Moreover, we have investigated whether BAR internalization occurs in the CNS. Purified coated vesicles isolated from bovine brain were found to contain BAR which was uncoupled to the GTP binding protein and adenylate cyclase. These BAR sites were labeled by a liphophilic ligand ^{125}I-cyanopindolol but not by a hydrophilic ligand ^{3}H-CGP-12177. This data suggest that BAR may be internalized by coated vesicle-mechanisms in the CNS. Thus, BAR internalization might play an important role in the CNS plasticity.

GPO 914-918

Our previous studies in the system of frog erythrocytes have provided first evidence suggesting that down regulation of BAR induced by agonist exposure is associated with internalization of BAR binding sites; these internalized BAR sites are present in the 30,000 x g supernatant of erythrocyte homogenate. Recent data show that these sites are associated with vesicular structures of more than 20 x 10^6 daltons (excluded by Sepharose 4B column chromatography). Moreover, these BAR sites can be readily labeled in a temperature dependent manner by lipophilic ligands such as dihydroalprenolol, cyanopindolol and hydroxbenzylpindolol but are labeled only with extremely low affinity by hydrophilic ligands such as CGP-12177 and isoproterenol. This suggests that these internalized BAR sites are present in inside-out endocytotic vesicles.

In order to get a better understanding of the molecular details involved in BAR internalization, we have initiated a cooperating project using antiserum raised against guinea pig lung BAR (provided by Dr. Craig Malbon) to stain BAR in frog erythrocytes (histology was done by Dr. Hannu Alho). The immunoblotting showed an excellent cross-reactivity between the antiserum and erythrocyte BAR which displayed a sharp band with Mr of 65,000-67,000. To make visible BARs present in intact erthrocytes, control and isoproterenol-treated cells were fixed with formaldehyde/glutaraldehyde and the fixed cells then incubated with the anti-BAR serum and further incubated with goat anti-rabbit Ig G conjugated with Biotin Avidin-gold. We found that about 50-60% of the control cells showed small, punctate dots after staining with anti-BAR serum. The number of punctates made visible under these conditions using control cells varied from 2 to 40. The staining could be shown to be localized mainly on the cell surface by careful focusing. The permeabilization of the control cells with saponin did not alter significantly the staining pattern or the apparent number of punctates in these cells. After 4 hr exposure of the cells to the BAR agonist ligand, isoproterenol, the density of the staining was markedly increased; the number of punctates was found to be increased to 100-150 per cells. The majority of cells were labeled by anti-BAR serum, while 10-20% of the cells showed no significant staining under these conditions. The permeabilization of desensitized cells with saponin further increased the staining to about 200 punctates per cell. By carefull focusing, the majority of the punctates appeared to be localized in the cytoplasmic regions of desensitized cells. Preimmune serum (or anti-insulin serum) showed no detectable staining on cells pretreated with isoproterenol and permeabilized with saponin.

The isoproterenol-induced alteration of BAR staining was found to be time-dependent. Little or no alternation in the staining was detected after 15 min of exposure to isoproterenol. The enhanced staining became more apparent at one hr following exposure to the agonist and reached a maximun after 4 hr. This time-course is similar to that for the desensitization and down-regulation of BAR in frog erythrocytes. In contrast to these effects of exposure of the cells to agonist, treatment of erythrocytes for 4 hr with the BAR antagonist, alprenolol, did not change the staining pattern or density obtained with anti-BAR serum. Alprenolol did abolish, however, the enhanced staining observed in cells exposed to isoproterenol alone. Taken together, these studies provide the first morphological evidence that BARs are internalized in cells exposed to chronic stimulation by BAR agonist.

To gain further insight on the nature of BAR in desensitized frog erythrocytes, we assessed the binding characteristics of the BARs previously shown to localize in a cytosolic fraction derived from desensitized cells. These BARs observed in a cytosolic fraction of desensitized cells were labeled in a temperature-dependent manner by the membrane-permeable, BAR antagonist ligand, ^{125}I-cyanopindolol (CYP). The more hydrophilic BAR antagonist, ^{3}H-CGP12177 is not able to permeate cell membranes and failed to label to any significant

extent BAR in this cytosolic fraction. Both radio-ligands labeled BARs equally well in plasma membrane fractions prepared from 30,0000 x g pellets of homogenized erythrocytes. The BARs recovered in the cytosolic fractions of desensitized cells migrated in the void volume of a Sephadex G-50 or Sepharose 4B column as measured by ^{125}I-CYP binding. These BARs appear to be associated with "inside-out" cytoplasmic vesicles which behave as particles with M_r greater than $20x10^6$.

It is still unknown as to whether internalization of BAR occurs in the CNS. Since, clathrin-coated vesicles (CVs) have been implicated in both endocytotic and intracellular transport of a variety of receptors, in collaboration with Dr. C.J. Coscia at St. Louis University, we have isolated CVs from bovine brain and examined them for the presence of BAR binding and adenylate cyclase (AC) activities. Microsomal pellets were subjected to linear D_2O/Ficoll gradient centrifugation and the resulting CVs enriched (60-80%) fraction applied to a controlled pore glass bead column to achieve further purification. The two major peaks of protein eluting from the column were monitored by electron microscopy and SDS-polyacrylaminde gel electrophoresis. Peak II contained almost exclusively CVs, whereas Peak I which appeared in the void volume contained larger smooth vesicles and few CVs. ^{125}I-CYP was found to bind specifically to sites in both peaks I and II with a Bmax of 28±4 and 32±3 fmol/mg protein, respectively. Binding of ^{125}I-CYP to both fractions was displaced by various BAR agents in a stereospecific manner. The addition of 50 uM Gpp(NH)p did not affect the displacement of CYP binding to Peak II sites by (-) isoproterenol, whereas a significant right shift was noted when Peak I or a synaptic plasma membrane preparation (SPM) from bovine hippocampus was used. ^{3}H-CGP-12177, a hydrophilic BAR ligand, specifically bound to SPM and to a lesser extent to Peak I, but failed to label the BAR present in Peak II, suggesting that receptors present in CVs were cryptic in nature. Rechromatography of Peak II on the glass bead column revealed that appreciable amounts of protein, CYP binding and adenylate cyclase were recovered in Peak I; this change in chromatographic migration was facilitated by pre-exposure of CVs to 0.5 M Tris, a condition known to cause at least partial dissociation of clathrin from these vesicles. These results suggest that at least part of the protein and BAR binding sites in Peak I were derived from CVs, possibly due to the loss of clathrin. Our results also suggest that BAR present in brain CV preparations might be molecular entities undergoing endocytotic or intracellular transport. We are currently investigating both possibilities.

It is well established that BAR in the CNS and peripherals can be up or down-regulated when the neuronal sympathetic activities fluctuate in vivo. It has been suggested that this receptor modulation is of a physiological and pharmacological importance. For example, down regulation of BAR induced by chronic antidepressant treatment is believed to be related to the therapeutic action. Up-regulation of BAR associated with hyperthyroidism and propranolol withdrawal syndrome, and down-regulation of BAR associated with hypothyroidism and hypoxia are most likely relevant to these disease states. The study using the system of frog erythrocytes has suggested that internalization of BAR is a major mechanism for the receptor down-regulation. Moreover, the detection and characterization of BAR in coated vesicles purified from bovine brain implies that internalization also takes place in the CNS. Thus, it seems reasonable to surmise that receptor down regulation is due to the acceleration of internalization whereas receptor up-regulation may be the result of deacceleration of this metabolic event. One of the proposed future studies is to elucidate the molecular details of BAR internalization using the BAR antibody to visulize the receptor at the electromicroscopic level. This study may lead to development of new drugs for the treatment of some diseases associated with abnormalities of the level of BARs.

Publications:

Chuang, D.-M., Dillon-Carter, O., Spain, J.W., Laskowski, M.B., Roth, B.L. and Coscia, C.J. Detection and characterization of beta-adrenergic receptors in coated vesicles isolated from bovine brain. J. Neuroscience. 6. 2578-2584, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01555-07 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enkephalin Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B. Mellstrom	Visiting Fellow	LPP-NIMH
H.-Y.T. Yang	Section Chief	LPP-NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated because the prinicipal investigator has left.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description: Z01 MH 01555-07 SMRP

The overall objective of this project is to investigate the enzyme(s) involved in the metabolism of met^5-enkephalin-arg^6-phe^7, and furthermore to search for potent enzyme inhibitors in an attempt to prolong the biological half life of this endogenous opioid peptide.

Previously, we have demonstrated that met^5-enkephalin-arg^6-phe^7 is rapidly metabolized by a dipeptidyl carboxypeptidase and an aminopeptidase; these two enzymes can be efficiently inhibited by Captopril and Hoe 498 (dipeptidyl carboxpeptidase inhibitors generally known as angiotensin converting enzyme inhibitors) and Bestatin (an aminopeptidase inhibior). Hoe 498 inhibits the met^5-enkephalin-arg^6-phe^7 degradation mediated by the carboxpeptidase with an IC_{50} value of 0.8 nM. Because of this high potency, in the present study, we have decided to investigate the sepcificity of Hoe 498 in potenciating the biological half life of met^5-enkephalin-arg^6-phe^7. Specifically, the effect of Hoe 498 on 1) the recoveries of met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8 released by 57 mM KCl from brain slices and 2) the analgesic potencies of met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8 were studied.

Methods and Findings:

A. Effect of Hoe 498 on the recoveries of released met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8.

Striatal slices prepared from fresh rat brains were perfused with an oxygenated Krebs-bicarbonate buffer containing 0.05% BSA at 37°C. The inhibitors to be tested and 57 mM KCl were added into the perfusion medium. The met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8 released were extracted and then determined by radioimmunoassays. The opioid peptides released were further identified by high pressure liquid chromatogaphy.

The recovery of the released met^5-enkephalin-arg^6-phe^7 induced by 57 mM KCl was increased in the presence of Hoe 498 while the released met^5-enkephalin-arg^6-gly^7-leu^8 was not protected by this inhibitor.

B. The effect of Hoe 498 on the analgesic potencies of met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8.

The analgesic effects of met^5-enkephalin-arg^6-phe^7 and met^5-enkephalin-arg^6-gly^7-leu^8 were studied in rats. Enzyme inhibitors and peptides were administered intraventricularly and the nociceptive responses were measured by tail flick latencies elicited by radiant heat.

Pretreatment of the rat with Bestatin (aminopeptidase inhibitor) resulted in potentiation and prolongation of the analgesic effect of met^5-enkephalin-arg^6-phe^7. Pretreatment with the combination of Bestatin and Hoe 498 further potentiated this analgesic effect. The analgesic effect of met^5-enkephalin-arg^6-gly^7-leu^8 was also potentiated by Bestatin, however, the combination of Bestatin and Hoe 498 failed to further increase this analgesic effect. The result suggests that met^5-enkephalin-arg^6-gly^7-leu^8 is not metabolized by the dipeptidyl carboxpeptidase, thus the potency of this peptide was not affected by Hoe 498.

We have previously observed that met^5-enkephalin is not protected by a dipeptidyl carboxpeptidase inhibitor. This observation and the results of present study suggest that Hoe 498 can be used to prolong the half life of met^5-enkephalin-arg^6-phe^7 specifically.

Significance to Biomedical Research:

It is now known that met^5-enkephalin-arg^6-phe^7 is rapidly degraded by the dipeptidyl carboxpeptidase and the aminopeptidase in vivo and potent inhibitors are needed to explore the physiological role of this peptide. Hoe 498, the highly potent dipeptidyl carboxypeptidase, in combination with an aminopeptidase inhibitor may be now used to better study the possible physiological role of the endogenous opioid peptide, met^5-enkephalin-arg^6-phe^7.

Proposed Course:

This project has been terminated because Dr. Mellstrom has left.

Publications:

Mellstrom, B., Iadarola, M.J., Yang, H.-Y.T. and Costa, E.: Inhibition of met^5-enkephalin-arg^6-phe^7 degradation by inhibitors of dipeptidyl carboxypeptidase, J. Pharmacol. Exp. Ther. 239:174-178, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01559-06 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Phe-Met-Arg-Phe-NH_2 Like Peptides In The Brain And Spinal Cord: Function And Distribution

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H.-Y. T. Yang, Ph.D.	Section Chief	LPP-NIMH
E. A. Majane	Chemist	LPP-NIMH
A.M. Alho	Visiting Fellow	LPP-NIMH

COOPERATING UNITS *(if any)*

1) Pertti Panula, M.D., Dept. of Anatomy, University of Helsinki, Finland
2) B.L. Roth, Surgical Research Division, Naval Medical Research Institute, Bethesda, MD

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously isolated from bovine brain and chemically characterized two putative endogenous opiate antagonist peptides. These peptides, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2, were found to be highly localized in periaqueductal gray and dorsal spinal cord, areas important for opiate antinociception. In this study, distribution and pathways of these two peptides in rat spinal cord were studied in detail by radioimmunoassay and immunohistochemistry. Levels of these two peptides are highest in dorsal gray and lowest in ventral white. After spinal cord transection, levels of these two peptides decreased significantly caudal to the transected region indicating the presence of descending pathways of these two peptides. Immunohistochemically, intense immunoreactivity was observed in substantia gelatinosa of posterior horn, scattered fibers were found in deeper laminae of posterior horn. The morphological study further suggests the possible role of these two peptides in opiate mediated analgesia.

In further studying the biological property of these two peptides, they were found to elevate mean arterial blood pressure. This pressor activity was attenuated by prior treatment with guanethidine or prazocin. The results suggest that these two peptides increase mean arterial pressure by potentiating the release of catecholamines.

The proposed course of this study is to investigate the mechanism by which these two peptides act to modulate opiate analgesia including release of these two peptides from spinal cord and receptors for these two peptides.

PHS 6040 (Rev 1/84)

GPO 914-918

In our earlier work, two peptides detected originally by the antiserum raised against Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2) were purified from bovine brain and chemically characterized. These two peptides, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2 (A-18-F-NH_2), can decrease tail flick latencies and F-8-F-NH_2 can also attenuate analgesia induced by morphine. These two peptides are highly localized in dorsal spinal cord and periaqueductal gray. These observations taken together suggest a possible role for these peptides in analgesia. In order to further explore the physiological role of these two peptides in the spinal cord, their distribution and pathways in rat spinal cord were studied in detail by radioimmunoassay and immunohistochemistry with specific antisera. These two peptides are unevenly distributed dorso-ventrally in the spinal cord; the levels are higher in gray than in white matter and the highest values are found in the dorsal horn. For the immunohistochemical study, sections of spinal cord were immunostained with the peroxidase/antiperoxidase method. Intense immunoreactivity was observed in the substantia gelatinosa of the posterior horn with antisera against F-8-F-NH_2. Scattered fibers were found in deeper laminae of posterior horn while only single occasional fibers were seen in ventral horn. The morphological study further suggests the possible role of these peptides in opiate mediated analgesia. The neuronal pathways of F-8-F-NH_2 in spinal cords were studied by lesion techniques. Seven days after a transection at thoracic level, there was a 50% decrease in F-8-F-NH_2 immunoreactivity caudal to the lesion while enkephalin, known to be interneuronal, was unchanged. Eight days after dorsal rhizotomy, F-8-F-NH_2 immunoreactivity was unchanged compared to sham operated while substance P levels in lesioned segments decreased by 35%. HPLC fractionation followed by radioimmunoassay failed to detect F-8-F-NH_2 immunoreactivity in sensory ganglia. The results suggest that at least part of the F-8-F-NH_2 immunoreactivity originates at the supra-spinal level.

In further exploring the biological property of F-8-F-NH_2 and A-18-F-NH_2, we have investigated the cardiovascular effect of these two peptides. Both peptides were found to elevate mean arterial blood pressure at 5-15 nmol/kg levels when injected into jugular vein. Furthermore, this pressor activity was attenuated, but not abolished, by prior treatment with guanethidine or prazocin. The results suggest that F-8-F-NH_2 and A-18-F-NH_2 elevate mean arterial pressure by potentiating the release of catecholamines and by mechanisms independent of catecholamine release.

To understand mechanism by which F-8-F-NH_2 acts to modulate opiate induced analgesia, affinity of F-8-F-NH_2 to opiate receptor was studied. F-8-F-NH_2 displaced ^3H naloxone from binding only at a very high concentration (10^{-5}M) suggesting that F-8-F-NH_2 functions differently from naloxone.

Significance to the Biomedical Research:

It is well established that endogenous opiate system exists. The evidence is now accumulating to indicate the existence of endogenous opiate antagonist system. The peptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) which is capable of attenuating opiate induced analgesia can function as one of the endogenous antiopiate peptides. By further studying the regulation of this peptide and mechanism of its action, we hope to better understand the pain perception and also the development of opiate tolerance.

Project Description: Z01 MH 01559-06 SMRP

Proposed Course of Study:

In order to understand the mechanism by which the peptide, Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) acts as antiopiate, we are planning to study (1) regulation of F-8-F-NH_2 release from spinal cord and (2) receptors including possible second messenger for F-8-F-NH_2 and A-18-F-NH_2.

Publications:

Majane, E.A. and Yang, H.-Y. T.: Distribution and Characterization of Two Putative Endogenous Opioid Antagonist Peptides in Bovine Brain. Peptides, (in press).

Roth, B.L., Disimone, J., Majane, E.A. and Yang, H.-Y. T.: Elevation of Arterial Pressure in Rats by Two New Vertebrate peptides FLFQPQRF-NH_2 and AGEGLSSPFWSLAAPQRF-NH_2 which are Immunoreactive to FMRF-NH_2 Antiserum. Neuropeptides, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01577-04 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Serotonin Pre- and Postsynaptic Components in NCB-20 Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J.-M. Cossery	Visiting Fellow	LPP-NIMH
X.-Z. Zhu	Visiting Fellow	LPP-NIMH
O. Dillon-Carter	Chemist	LPP-NIMH
D.-M. Chuang	Group Chief	LPP-NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Immunochemistry Group

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.6	1.6	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used NCB-20, a cloned cell line of mouse neuroblastoma and fetal chinese hamster brain cell, as a model system to study receptor interactions in the same cell. NCB-20 cells express serotonin (5-HT) receptors coupled to adenylate cyclase. We found that 5-HT and 5-methoxytryptamine activated adenylate cyclase in intact cells with low efficiency (EC_{50} values ~500 nM), while some putative 5-HT_{1A} receptor agonists, 8-hydroxy-2-(di-n-propylamino) tetralin or 8-OH-DPAT, ipsapirone and buspirone were ineffective. Receptor binding studies also showed that 5-HT was bound to NCB-20 cell membranes with low affinity, while vitrually no significant binding of 8-OH-DPAT was detected. These data suggest that 5-HT receptor coupled to adenylate cyclase in NCB-20 cells is distinct from 5-HT_{1A} and may represent a novel class of receptors for 5-HT. We also found that NCB-20 cells are equipped with presynatic components of 5-HT neurons. These include a 5-HT uptake system and specific binding sites for imipramine which inhibits the uptake of 5-HT in a competitive manner. Addition of imipramine activated phosphosphoinositide hydrolysis catalyzed by phospholipase C. This activation was dose-and time-dependent and was nonadditive to that produced by carbachol. Moreover preexposure to imipramine induced a rapid desensitization to the response of imipramine and carbachol. Since NCB-20 cells possess multiple receptors coupled to different effector systems, this cell line is an usual system to study receptor cross-talks at the molecular levels.

GPO 914-918

Project Description: Z01 MH 01577-04 SMRP

The understanding of the molecular mechanisms of the interactions between receptors for neurotransmitters in CNS has been hampered by the complexity of brain structures. Part of this complexity arises from the presence of heterogenous cell populations which include not only neurons but also glial cells. This understanding may be facilitated by the use of a model system of a cloned cell line which contains multiple receptors for neurotransmitters. We have found that NCB-20, a cloned hybrid cell line of mouse neuroblastoma and fetal Chinese hamster brain cell could be such a model system.

In confirming the observations by Nirenberg and coworkers (MacDermot et al., PNAS 76:1135-1139, 1979), we have found that NCB-20 cells have 5-HT sensitive adenylate cyclase. We have extended this finding by characterizing the 5-HT receptors coupled to adenylate cyclase. 5-HT receptors can be divided into 5-HT_1 and 5-HT_2. 5-HT_1 receptors bind 5-HT with high affinity (low nanomolar Kd) but are relatively insensitive to the classical 5-HT antagonist (micromolar affinity). In contrast 5-HT_2 receptors bind 5-HT with low affinity (micromolar affinity) but are highly sensitive to the classical 5-HT antagonist (nanomolar affinity). 5-HT_1 receptors can be further subclassified into 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}. It is currently believed that 5-HT_{1A} is coupled to adenylate cyclase. We found,, however, that 5-HT and 5-methoxytryptamine activated adenylate cyclase in intact NCB-20 cells with low affinity; their EC_{50} values were approximately 500 nM. Some putative 5-HT_{1A} receptor agonists, 8-hydroxy-2-(di-n-propylamino) tetralin or 8-OH-DPAT, ipsapirone and buspirone were totally ineffective in activating adenylate cyclase activity. Receptor binding studies also showed that 5-HT was bound to NCB-20 cell membranes with low affinity but no specific binding of 8-OH-DPAT was detected. These results suggest that 5-HT receptor coupled to adenylate cyclase in NCB-20 cells is distinct from 5-HT_{1A} receptor. Recently it was reported by Shenker et al (Molecular Pharmacol. 31, 357-367, 1987) that in hippocampal membranes, 5-HT activates the activity of adenylate cyclase biphasically. While the high affinity componeut ($EC_{50} \sim 20$ nM) is due to activation of 5-HT_{1A} receptor, the nature of this low affinity component ($EC_{50} \sim 400$ nM) is unknown. It is conceivable that NCB-20 cells express a homogenous population of this low affinity 5-HT receptor which represents a novel subtype previously unclassifed.

Interesting enough, NCB-20 cells also possess presynaptic components of 5-HT neurons (Nakaki et al., J. Neurochem. 45: 920-925, 1985). These include a 5-HT uptake system and a high affinity binding site for imipramine, a classical tricyclic antidepressant which inhibits 5-HT uptake. Studies by us and others in the CNS have suggested that imipramine is bound to a presynaptic site controlling the uptake of 5-HT in a negative manner. It is of important to mention that the density of imipramine binding site in NCB-20 cells is very high (~ 20 pmol/mg protein). We have recently found that addition of imipranine to NCB-20 cells increased the hydrolysis of phosphoinositide catalyzed by phospholipase C. In these experiments, cells were preincubated with ^{3}H-myo-inositol to label the endogenous inositol phospholopids and the hydrolysis of phosphoinositide was expressed as the accumulation of ^{3}H-inositol monophosphate in the presence of lithium. The EC_{50} of imipramine for this response was about 20 uM and the saturating concentration was 100 uM. The increased hydrolysis of phosphoinositide produced by imipramine was nonadditve to that induced by carbachol, a muscarinic cholinergic receptor agonist. However the imipramine response was unaffected by antagonists for muscarinic cholinergic, alpha$_1$-adrenergic, histaminergic H_1 and 5-HT_2 receptors. Preexposure of NCB-20 cells to 20 uM imipramine caused a time dependent desensitization to subsequent stimulation with imipramine or carbachol. These data suggest that imipramine "receptors" and muscarinic cholinergic receptors may be coupled to the same pool of phosphoinositide which is the substrate for phospholipase C.

Because NCB-20 cells are equipped with many different types of neurotransmitter receptors and drug binding sites, this cell line is ideal for the study of multiple receptor interactions at the functional and biochemical levels. The information obtained from this model system may lead to a better understanding of the communication between receptors in the same cell and eventually provide a rational basis for a new approach to the development of therapy for some mental illnesses which are related to abnormalities of receptor-receptor interactions.

Our future plans are to fully characterize the nature of the 5-HT receptor subtype coupled to adenylate cyclase in NCB-20 cells. We are also attempting to elucidate the role of imipramine and muscarinic cholinergic receptor-coupled phospholipase C in this model system.

Publication:

Chuang, D.-M. Carbachol-induced accumulation of inositol-1-phosphate in neurohybridoma NCB-20 cells: Effects of lithium and phorbol esters. Biochem Biophy. Res. Commun. 136:622-629, 1986.

Roth, B.L. and Chuang, D.-M. Multiple mechanisms of serotonergic signal transduction.Life Science (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01578-04 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Genes for Insulin in Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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SECTION

Group of Immunochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It has been suggested that insulin-like proteins may be growth factors in the nerous systems. The aims of this project are to use a cDNA probe to detect the presence of mRNA for insulin or insulin-like peptide in extrapancreatic tissues including the brain and to investigate their physiological role. We extracted RNAs from various tissues using liquid nitrogen pulverization and by homogenization in the presence of GuSCN. The RNA pellets recovered from CsCl-cushion following centrifugation of the homogenate are subjected to oligo-dt columns for the purification of poly A$^+$RNAs which include mRNAs. These isolated mRNAs are electrophoresed on agarose gel followed by blotting to a nitrocellulose membrane. These immobilized mRNAs are then hybridized to a cloned cDNA fragment of proinsulin gene which has been nick-translated with ^{32}P-dCTP. We found that the ^{32}P-cDNA probe is hybridized to mRNA from extrapancreatic tissues under stringent conditions (i.e., high temperature and low salts). However, the molecular sizes of these hybridizable mRNA transcripts are different from that detected in the pancreas. Thus, the size of pancreatic mRNA is of 0.5 kilobase, whereas two major species of mRNA transcripts detected in the gut, heart and to a lesser extent, liver have approximately 4.2 and 2.2 kilobases. We also detected these two mRNA transcripts in the brain and a cloned cell line NCB-20 (neuroblastoma x fetal hamster brain cell hybrid), suggesting a neuronal location of these transcripts. Some minor mRNA species hybridizable to the cDNA probe were also found in the brain and other tissues. Thus, mRNA for insulin and insulin-like peptides can be detected in extrapancreatic tissues including the brain. We are current attempting to study the role of these insulin-like peptides in the growth, maturation and survival of neurons.

GPO 914-918

Project Description: Z01 MH 01578-04 SMRP

It has been reported that insulin and insulin-like growth factors are widespread throughout the central nervous systems (Havrankova et al., Nature 272:827, 1978). However, neither the nature nor the origin for these polypeptides has been clearly defined. In fact, because of the low quantity of insulin (defined by reactivity with an insulin antibody), it has been suggested that brain insulin is blood borne. The present study was designed to assess this question using a cloned cDNA fragment of proinsulin (kindly provided by Dr. Villka-Komaroff, Univ. of Massachusetts) to examine for the presence of mRNAs in the rat bain and other extrapancreatic tissues that can be hybridized to this cDNA probe.

We isolated Poly A^+RNAs (which include mRNA) from the tissue and then electrophoresed on an agarose gel followed by blotting to a nitrocellulose membane. These immobilized mRNAs are then hybridized to the proinsulin cDNA which has been nick-translated with ^{32}P-dCTP. We found that the ^{32}P-cDNA probe is hybridized to mRNAs from several extrapancreatic tissues under stringent conditions (42^{o}C with 0.8 N NaCl and 50% formamide). However, the molecular sizes of these hybridizable transcripts are different from that detected in the pancreas; the size of pancreatic hybridizable mRNA is approximately 0.5 kilobase, whereas two species of RNA transcripts hybrized to probe are detected in the gut, heart and to a lesser extent, the liver. A rough estimation of their size using ribosomal RNAs as the marker reveals that they have 4.2 and 2.2 kilobases. Interestingly, we also found that there are two species of hybridizable RNA transcripts in the brain with approximately the same size. Moreover, these two RNA transcripts are also detected in cultured cells of NCB-20 which is a cloned line of mouse neuroblastoma x fetal Chinese hamster brain cell, suggesting that these transcripts in the brain are of neuronal origin. Some minor species of mRNA with sizes between 4.2 and 2.2 kilobases in the brain and other tissues were also found to be hybridized with the cDNA. Since insulin (or insulin-like peptide) is detectable in the brain, one may infer that these brain RNA transcripts are translated into proteins. However, the nature of these proteins is currenlty unknown. It should be stressed that the level of these RNA transcripts in extrapancreatic tissues is at least 50 times lower than that of proinsulin mRNA in the pancreas. The low abundance may explain the failure of previous investigators to detect their presence in these extrapancreatic tissues of adult rats.

We have shown previously that the addition of insulin to olfactory bulb slices of rat modulates the production of cAMP that is increased by dopamine (Barbaccia et al., Regulatory Peptides: From Molecular Biology to Function, pp 511-518, 1982). It is possible that insulin or insulin-like peptides encoded by the mRNA transcript detected in the present study may function as a neuromodulator. In light of the growth promotion activity endowed with insulin-like growth factor in some systems, it is also likely that these putative brain insulin-like peptides are nerve tropic factors required for the maturation of neurons. In support of the view is the recent finding that insulin-like growth factor I promotes the synthesis of DNA in both neuronal and glial cells (J. Biol. Chem. 262, 7693-7699, 1987). The purposed course of this project to further characterize the insulin-like proteins encoded by the two mRNA species detected in the present report and to elicit their role in the growth maturation and survival of neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01579-04 SMRP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of an Endocoid for the $5HT_2$ Recognition Site

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B.L. Roth	Principal Investigator	Naval Medical Res. Inst.
D.-M. Chuang	Chemist	LPP-NIMH
X.-Z. Zhu	Visiting Fellow	LPP-NIMH

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

$5HT_2$ receptors have low affinity for serotonin (5-HT) and do not show receptor supersensitivity following lesion of nerve ending. We have proposed for the existence of an endogenous ligand (endocoid) for this receptor site. Our previous studies have demonstrated that some protein fractions derived from bovine and rat brains display activity of inhibiting ^{3}H-ketanserin binding but inhibiting only slightly ^{3}H-mianserin binding to rat brain membrane. This protein (Mr=6000) has been partially purified by gel filtration and HPLC column chromatography.

The present studies show that two ^{3}H-ketanserin recognition sites are present in rat striatum as revealed by autoradiography and nonlinear, least square regression algorythm. The high affinity site (Kd 0.4 nM) is similar to the 5-HT_2 site previously characterized by various investigators. The low affinity site (Kd 20 nM) has a unique pharmacological specificity and is preferentially localized to rat striatum and septum. A series of conventional 5-HT_2 receptor antagonists, 5-HT and 5-HT uptake blockers are ineffective at inhibiting ^{3}H-ketanserin binding to this low affinity site. Also, chronic treatment with p-chlorophenylalanine, which deplets brain 5-HT, up-regulates only the high affinity site. Moreover, selective lesion of 5-HT nerve ending with a neurotoxin failed to affect this low affinity binding sites. Thus, in the striatum and septum, ^{3}H-ketanserin labels a unique recognition site. This site has recently been shown to be associated with dopaminergic nerve endings and may regulate biogenic amine release through recognition by some endogenous ligand. The present findings may shed light on the role of this peptide endocoid in the modulation of neurotransmission.

PHS 6040 (Rev. 1/84)

GPO 914-918

The serotonin system has been shown to be involved in the action of certain antidepressant drugs. There may exist at least two classes of serotonin (5-HT) recognition sites in mammalian brain. These sites have been designated 5-HT_1 and 5-HT_2 and show differential pharmacologic specificity. In brief, the 5-HT_1 site binds ^{3}H-5-HT with high affinity (kd in nM ranges), is regulated by guanine nucleotides and may be coupled to adenylate cyclase. The 5-HT_2 site binds 5-HT with somewhat lower affinity (kd 20 uM), is not regulated by guanine nucleotides and binds certain 5-HT antagonists (e.g., ketanserin and mianserin) with very high affinity (kd in nM ranges). In addition, the 5-HT_2 recognition site appears to mediate certain behavioral effects and peripheral vascular contraction caused by 5-HT. We have recently shown that 5-HT_2 receptor recognition sites in rat aorta are linked to phosphoinositide hydrolysis by phospholipase C. Using ^{3}H-mianserin and ^{3}H-ketanserin as ligands, we have previously shown that these two compounds might be labelling distinct recognition sites. Since pharmacological manipulations which would be expected to result in supersensitivity of 5-HT_2 recognition sites (e.g. lesioning of 5-HT nerve terminals) affected only the ^{3}H-mianserin recognition site, it was suggested that 5-HT might not have been the major endogenous ligand for the 5-HT_2 recognition site. We have therefore searched for an endocoid for the 5-HT_2 recognition site and have identified this compound in the brains of rat and bovine.

We have previously reported that in the brain there exists an endogenous polypeptide ligand that displays the activity of inhibiting ^{3}H-ketanserin binding to the rat brain membane. To a lesser extent and with a weaker potency, this protein also inhibits the binding of ^{3}H-mianserin to the membrane preparation but does not affect the receptor binding to 5HT_1, B-adrenergic receptors and imipramine binding sites. This peptide has been partially purified by selective acid extraction, CM-Sephadex, Biogel p-10 and reversed phase HPLC column chromatography and appears to have a Mr of about 6000 daltons.

In the present study we have attempted to characterize an unique ^{3}H-ketanserin recognition site in the rat striatum which could be the receptor site for this endogenous peptide. ^{3}H-ketanserin binding was measured in crude membrane preparations from rat striatum or frontal cortex in 50 mM Tris-HCl buffer (pH=7.40 at 25^{o}C). Binding isotherms consisting of 12-24 concentrations of unlabelled ligand were analyzed by the LIGAND program (Munson and Rodbard, 1981) while autoradiographic studies were performed as described by Herkenham using cryostat sections and LKB-Ultrofilm. In horizontal sections of rat brain, ^{3}H-ketanserin (1.0 nM) intensely labelled layer IV of the cortex, the striatum and septum. In the presence of 100 nM mianserin we found that the cortical sites were displaced, while the striatum and septum were unaffected.

Our radioligand binding assays of rat striatum disclosed high (k_d=0.39$\pm$.01 nM;Bmax=104 fmole/mg) and lower (k_d=21.8$\pm$7.2 nM;Bmax=2993 fmole/mg) affinity ketanserin binding sites. Classical 5-HT_2 antagonists had high affinity (low nanomolar k_d) for only the high affinity site and micromolar affinities for the lower affinity site.

Alpha$_1$-adrenergic, dopaminergic , histaminergic, and other agents were ineffective at the low affinity ketanserin site. Only the higher affinity sites were increased by chronic para-chloro-phenylalanine treatment (104$\pm$18 vs 175$\pm$13 fmole/mg) which depletes endogenous 5-HT. Moreover, selective lesion of 5-HT nerve terminals by the neurotoxin, 5,7-dihydroxytryptamine, failed to change the density of this low affinity ^{3}H-ketanserin binding sites. This low affinity site has recently been shown to be abolished by 6-OH-dopamine lesion of dopaminergic terminals (Leysen et al., Eur. J. Pharmacol. 134, 373-375, 1987). It is conceivable that the proposed endogenous ligand may recognize this low affinity ketanserin binding sites and modulate the release of dopamine from its nerve endings.

It has been shown that long term treatment with ketanserin causes a marked loss of dopamine content in the striatum. It is conceivable that this effect is mediated by the low affinity ketanserin binding sites shown in this report. This study also raises an interesting possibility that the endogenous ligand characterized in our earlier studies may participate in the modulation of the function of dopamine released from striatal nerve endings. Abnormalities in this putative endogenous ligands or its binding sites in the striatum might result in malfunction of dopamine neurons such as that found in Parkinsonism. Our future plans are to further characterize the nature of this endogenous ligand and to study the regulation of this unique striatal ketanserin binding sites.

Publications:

1. Roth, B.L., McLean, S., Zhu, X.-Z. and Chuang, D.-M. Characterization of two ^{3}H-ketanserin recognition sites in rat striatum. J. Neurochem. (in press).

2. Roth, B.L. and Chuang, D.-M. Multiple mechanisms of serotonergic signal transductions. Life Sci. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01584-04 SMRP

PERIOD COVERED
October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Noncompetitive Interactions Between Mu- and Delta-Opiate Receptors in Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard B. Rothman	Guest Researcher	LPP-NIMH
M. Herkenham	Staff Scientist	LNP-NIMH
S. McLean	Staff Fellow	LNP-NIMH
J. Cadet	Staff Scientist	NPB-NIMH
J. Byrd	Guest Researcher	LPP-NIMH
K. Rice	Staff Scientist	LC-NIDDK
V. Bykov	Student Volunteer	LPP-NIMH

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SECTION
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NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Work in my laboratory is directed towards testing a model of the opioid receptors sufficiently complex enough to explain varied physiological data. This model postuates a receptor complex consisting of adjacent and interacting mu, delta and kappa binding sites, as well as distinct mu, delta and kappa receptors not associated with the receptor complex.

To test this model we utilize several techniques: (1) quantitative ligand binding studies using the method of "binding surface analysis" and weighted nonlinear least squares curve fitting, (2) site-directed alkylating agents such as BIT (mu-directed), FIT (delta-directed) and beta-FNA (mu-directed), (3) receptor autoradiography to provide anatomical information, (4) "in vivo" manipulations such as chronic morphine and chronic naltrexone which up-regulate opiate receptors and (5) biochemical information using a technique to cross-link ^{125}I-beta endorphin to opiate receptors.

As our work progress, not only is the model tested, but additional data is generated. Our work addresses fundamental issues of morphine tolerance and dependence. It has defined the opiate receptors labeled by (^{3}H) cycloFOXY, a novel antagonist suitable for position emission tomography. We have developed methods for measuring rat brain kappa receptors, a subtype of the opiate receptor implicated in eating disorders and work is underway to develop a kappa-directed site-directed alkylating agent.

My laboratory conducts research in two main areas, and in collaboration with Dr. Byrd and Dr. Cadet and two others as well. These projects will be discussed in turn:

1. Evidence for an opiate receptor complex consisting of interacting mu, delta and kappa binding sites.

2. Effect of chronic morphine and chronic naltrexone on rat brain opiate receptors.

3. Effect of psychotropic drugs on rat brain PCP receptors and their relationship to sigma opiate receptors.

4. Alterations in CNS neurotransmitter receptors in an animal model of dyskinesia.

Project 1.

The primary goal of this project is to document and explore noncompetitive interactions between opiate receptors. Our hypothesis postulates a four receptor model. Postulated is a receptor complex consisting of adjacent and interacting mu, delta, and kappa binding sites. "Pure" mu, delta, and kappa sites not associated with the complex are also postulated.

The in vitro data supporting portions of this model is the phenomenon of noncompetitive inhibition. Thus, we have demonstrated that mu-ligands such as morphine are noncompetitive inhibitors of the lower affinity binding site labeled by the enkephalin analog [^{3}H]D-ala^2-D-leu^5-enkephalin ([^{3}H]DADL). We therefore infer that [^{3}H]DADL labels the delta site of the complex, and that the noncompetitive interactions is mediated through the adjacent mu binding site. We call this the mu-noncompetitive delta binding site.

We have also previously showed that leucine enkephalin noncompetitively inhibits a binding site labeled by [^{3}H]-naloxone, and therefore inferred that [^{3}H]-naloxone was lebeling the mu binding site of the complex, and that the noncompetitive inhibition was mediated via the adjacent delta site.

To test this model, we used several techniques which provide complementary information. This is essential, since all radiolabeled ligands we have used label at least two binding sites.

A. Quantitative ligand binding studies using the "binding surface" method and weighted nonlinear least squares curve fitting. The types of questions generated by this model calls for a highly refined and fine-tuned approach to conducting the opiate binding assay. The "binding surface" approach to experimental design significantly extends the types of data and experiments appoachable with receptor binding techniques.

B. Use of site-directed alkylating agents. These agents have been developed by Dr. Rice and associates. In the context of the model, they provide a means of selectively eliminating populations of opiate receptors, allowing the remaining ones to be studied in detail.

The two agents we have characterized in detail are 2-(p-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyanatobenzimidizole-HCl (BIT) and N-phenyl-N-[1-(2-(p-isothiocyanato)phenylethyl)-4piperidinyl]propanamide)-HCl (FIT). We have shown that BIT selectively eliminates all mu (mu_{cx} and mu_{ncx}) binding sites, leaving delta and kappa. FIT

on the other hand selectively eliminates the delta$_{ncx}$ or mu-competitive binding site. Thus, FIT-treated membranes possess only the delta$_{cx}$ (mu-noncompetitive) binding site. Consistent with detailed binding studies, we showed mu-ligands were noncompetitive inhibitors of [^{3}H]DADL binding to FIT-treated membranes.

In addition to using BIT and FIT to test the predictions of the model, they have proven invaluable in receptor localization studies and in studying the effect of chronic morphine and naltrexone on opiate receptors. This is because treatment of membranes with these agents allows unambiquous assay of selected opiate receptors.

LIGAND	AGENT	RECEPTOR
[^{3}H]Bremazocine	BIT+FIT	kappa
[^{3}H]DADL	BIT	delta$_{ncx}$
[^{3}H]DADL	FIT	delta$_{cx}$
[^{3}H]cycloFOXY	BIT	kappa$_{cx}$

Another site-directed alkylating agent we have studied in detail is beta-funaltrexamine (beta-FNA). This derivative of naltrexone has been shown to irreversibly block mu receptors in smooth muscle assays and in analgesic assays. We previously showed that FNA-treated membranes lost only a portion of their mu-binding sites (unlike BIT-treated membranes). Autoradiographic studies (conducted with M. Herkenham) have demonstrated that the FNA-sensitive and FNA-insensitive binding sites are differentially distributed across regions of the rat brain.

Our working hypothesis is that FNA was eliminating the mu$_{cx}$ binding site, and might be useful in vivo as an irreversible antagonist of the receptor complex. If this were the case, than FNA would be a powerful tool in determining the physiological functions mediated by this unique opiate receptor.

Our data to date support this hypothesis. Thus, we have shown that treatment of membranes in vitro with FNA, or in membranes prepared from rats injected i.c.v. with FNA 18 hr prior to sacrifice, the delta$_{cx}$ binding site is selectively eliminated without major changes in the binding of mu or kappa ligands. These data support the hypothesis that beta-FNA alkylates the receptor complex, and that the delta$_{cx}$ binding site and the mu binding sites are not identical. Portions of these studies were done in collaboration with Dr. Holaday.

C. Receptor localization studies were done in collaboration with M. Herkenham and S. McLean. For our purposes, the major use of autoradiography arises from the simple notion that if two binding sites possess different anatomical distributions, then they are probably distinct sites. If two sites are identically distributed across regions of the brain, then they might be distinct sites. Thus, our primary use of autoradiography is to test for "identity of binding sites".

Since all ligands label more than one site, localization studies of single binding sites requires determining conditions for selective single site labeling. To do so we have utilized slide mounted sections of molded minced rat brain. With this method, rat brain is made into a paste, molded in a test tube, and then frozen. Sections are cut in a cryostat. With this technique, each section is the same as the next, and large, thick sections can be used to increase the cpm bound. Using these sections, binding sites are characterized using "binding

surfaces," and the Kd's, Bmax's, and concentrations of blocking agents determined. Since we started using this method, it has become clear that although qualitatively similar data are obtained with membranes and sections, striking quantitative differences are more often the rule. Thus, it is essential to determine binding parameters with sections.

Using autoradiography, the squirrel hippocampus was identified as an excellent model system with which to test the model. In this species, most types of opiate receptors appear to have distinct anatomical distributions.

Autoradiographic studies were invaluable in determining the types of opiate receptors labeled by [^{3}H]cycloFOXY, a novel opiate antagonist suitable for PET studies, prepared in Dr. Rice's laboratory. Ligand binding studies showed that [^{3}H]-cyclo-FOXY labeled two sites, putatively identified as mu and kappa. Autoradiograms using guinea pig brain confirmed this, in that [^{3}H]-cycloFOXY labeled the deep layer of the cortex. Additional studies in the rat confirmed this, as i.v. injections of [^{3}H]-cycloFOXY labeled the neural lobe of the pituitary, a structure previously shown to possess only kappa sites. Similarly, it failed to label the corpus callosum, a structure which possesses only delta sites. Thus, autoradiographic studies allowed names to be given to sites defined on the basis of ligand binding experiments.

D. A fourth technique utilized for testing the model is "in vivo" manipulations. By this we mean administration of chronic drugs, followed by determination of which of the opiate binding sites are altered. In the context of examining the model, this method is used to test "identity of binding sites". For example, we have shown that chronic morphine selectively up-regulates the delta site of the delta$_{cx}$ binding site. We postulated that the [^{3}H]-antagonist binding site at which leu-enkephalin is a noncompetitive inhibitor is the mu-site of the complex. If this is true, then chronic morphine should also up-regulate this site. This is indeed the case. Thus, the mu-noncompetitive delta binding site and the LE-noncompetitive antagonist binding site are the same receptor. This work will be discussed in greater detail in the section describing project 2.

E. The 5th method we will be using [^{125}I]-beta-endorphin. This ligand is covalently linked to opiate receptors. The receptors are solubilized and subjected to SDA-gel electrophorsis, allowing visualization of individual bands. Work with this method, in collaboration with Dr. Bryan Roth, is underway, and should prove useful in testing aspects of the model.

Project 2

This project examines the effect of chronic morphine and chronic naltrexone on opiate binding sites labeled by various ligands. As discussed above, these experiments were initially designed as "identitiy of binding site" experiments. To date we have at this time is only partially complete and is tabulated below:

SITE	MORPHINE	NALTREXONE
Delta$_{cx}$	UP	UP
Delta$_{cx}$	NO CHANGE	UP

SITE	MORPHINE	NALTREOXNE
Mu_{cx}	UP	--
Mu_{ncx}	NO CHANGE	UP
Kappa	NO CHANGE	--

UP refers to up-regulation.
-- refers to results pending.
NO CHANGE means no detectable change.

The remainder of the table should be completed by the end of 9/87. The data will provide information concerning the relationship between the binding sites labeled with the different ligands. Additionally, aliquots of membranes have been saved and have been used in the cross-linking studies described above. The pending results should allow correlations between sites labeled on membranes and bands on the gel, as well as a means to check for molecular alterations in opiate receptors.

The finding that chronic morphine increases the density of certain populations of opiate receptors is intriguing. This finding in fact may be the first relevant biochemical marker for the development of tolerance and dependence. The fact that this change is detectable in whole brain membrances and the fact that we see an up-regulation rather than a down-regulation suggests the involvement of endogenous anti-opiate peptides. Perhaps morphine releases an endogenous antagonist which causes the up-regulation? If this peptide could be identified, then a means would be available to prevent tolerance and dependence while retaining the acute (and therapeutic) effects of opiates.

For this reason, the major goal of the project is to study the mechanism of the opiate-induced up-regulation. There are several endogenous peptides that are physiological opiate antagonists. These will be chronically administered i.c.v. The appropriate peptide should up-regulate the same spectrum of receptors up-regulated by chronic morphine. Further, co-administration of anti-IgG directed against the peptide with morphine should prevent the up-regulation as well as tolerance and dependence. This work will commence once the table above has been completed.

Project 3

This project is in part conducted with Dr. J. Byrd. We were first interested if the interaction of opiate drugs with PCP receptors was competitive or noncompetitive. If noncompetitive, then it might be possible to design drugs based on opiate structures which would have antagonist actions at the PCP receptor. A series of experiments demonstrated that opiates of several different chemical classes were all competitive inhibitors at the PCP receptor.

A role for PCP receptors in major mental disorders has been postulated by some investigators. As a preliminary study, we infused for 12 days several psychotropic medications including an MAO inhibitor, naloxone, haldol, and the potent opiate agonist etonidazine. Subcutaneously placed osmotic minipumps were used. The MAO inhibitor

significantly decreased the Kd of [^{3}H]-TCP, which was used to assay PCP receptors. Naloxone and etonidazine increased the Kd. This raises the interesting possibility that chronic treatment of humans with naltrexone will "desensitize" the PCP receptor. This straegy may be helpful in treatment resistant schizophrenics as well as abusers of PCP. Membrances from haldol treated rats await demonstrated a substantial increase in the Bmax of PCP receptors.

Project 4

This project is conducted in collaboration with Dr. Cadet, NSB. He has shown that treatment of rats with the chemical IDPN causes a persistent dyskinesia. Our lab has assisted him in determining possible alterations in CNS receptors in caudate, hippocampus, and cortex. We have shown a significant 25% decrease in striatal delta receptors. Work on measuring other receptors has been submitted for publication.

PROPOSED COURSE OF THESE PROJECTS .

Some aspects of our plans are described above. These will be summarized by project.

Project 1 - Model of the opiate receptors.

a. Using [^{3}H]bremazocine and [^{3}H]cycloFOXY and other alkylating agents to see if there are two kappa binding sites distinguished by noncompetitive interactions. A major goal of this study is to identify, in collaboration with Dr. Rice, a selective kappa receptor alkylating agent.

b. Examine the anatomical distribution of ligand binding sites in squirrel hippocampus and to check for "identity of binding sites." This model may provide clear cut anatomical evidence for two mu binding sites.

c. Continue to gather correlative data using the cross-linking method.

d. Complete the table describing the effects of chronic morphine and chronic naltrexone on opiate receptors for the "identity of binding sites strategy," and in collaboration with Dr. Holaday continue to work on the mechanism(s) of the chronic morphine induced up-regulation.

e. Continue working with Dr. Rice on a subtype-selective alkylating agent.

f. Finish characterizing the opiate receptors eliminated by FNA, and correlative studies using the cross-linking method.

Project 2

a. Complete the table as described above.

b. Having established which ligand gives the best signal in response to chronic morphine, show that the time course of the up-regulation parallels the development of tolerance and dependence.

c. 5,6-dihydroxytryptamine lesions of the raphe system block the development of tolerance and dependence. We will see if this lesion blocks the up-regulation.

d. Chronic i.c.v. administration of putative peptide opiate antagonists followed by assays of opiate receptors.

e. Localization of brain structures most affected by morphine using receptor autoradiography and comparison to naltrexone.

f. Cross-linking studies to look for molecular changes.

Project 3

1. Effect of chronic haldol on PCP receptors.

2. Effect of chronic naltrexone using dissected areas of rat brain instead of whole brain membranes.

Project 4

Since Dr. Cadet has left the NIMH, this collaboration has been terminated.

SIGNIFICANCE OF THIS WORK FOR BIOMEDICAL RESEARCH

The work described above crosses several disciplines. Our work with opiate receptors is of seminal importance to physiologists trying to decipher the actions of the many endogenous opioid peptides, particularly the ability of some opioid peptides to modify the actions of others. Our studies on the effect of chronic morphine on opiate receptors provides a relevant biochemical marker with which to study mechanisms of tolerance and dependence. In addition, our use of "binding surface analysis" has made possible fruitful collaborations in clinically relevant models of human disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01585-03 SMRP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Mechanisms of Smooth Muscle Cell Contraction in Rat Aorta

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B.L. Roth	Guest Researcher	LPP, NIMH
T. Nakaki	Visiting Fellow	LPP, NIMH
D.-M. Chuang	Group Chief	LPP, NIMH

COOPERATING UNITS *(if any)*

Naval Medical Research Institute, Surgical Res. Br., Bethesda, MD

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group of Immunochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.4	1.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The smooth muscle of rat aorta was used as a model for the study of the molecular mechanisms of 5-HT_2 receptor function. We have previously shown that 5-HT receptors in rat aorta are coupled to phosphoinositide (PI) - specific phospholipase C. Further, we showed that the mechanism of contraction elicited by 5-HT is a complicated scenario involving receptor-mediated activation of calcium channels and a phospholipase C. We now report that in rat aorta, the 5-HT-induced contraction and PI turnover are modulated by biologically active phorbol esters. In rat aorta, 5-HT stimulated PI turnover and contraction (EC_{50}=10±3 uM); these two responses were highly correlated (r=0.95; $P<.01$). We have characterized the inhibitory potency of a variety of 5-HT-antagonists against the stimulation of PI turnover elicited by 5-HT. Classic 5-HT_2 antagonists mianserin, ketanserin, metergoline and pizotifen were found to inhibit this response in the low nanomolar range; amitryptiline and haloperidol were 10- to 20- fold less potent. The alpha-1 receptor antagonist, prazosin, was inactive in micromolar concentrations. The potency of the 5-HT_2 antagonists was correlated with their ability to displace [^{3}H]ketanserin binding from rat frontal cortex membranes (r=0.90; $P<.05$). The tumor promoter phorbol dibutyrate was found to inhibit 5-HT-stimulated PI turnover at low nanomolar concentrations whereas the biologically inactive substance 4-α-phorbol was ineffective. Pretreatment of rat aorta with phorbol dibutyrate at concentrations that inhibited 5-HT-induced PI turnover also attenuated the aortic contraction induced by 5-HT in the presence of a calcium channel blocker nitrendipine. Our results suggest that phorbol esters may densensitize 5-HT_2-receptor-mediated PI turnover and contraction of rat aorta, possibly via an activation of protein kinase C.

GPO 914-918

Project Description: Z01 MH 01585-03 SMRP

Recent evidence suggests that extracellular informational signals in a variety of systems are transduced into the cellular interior through activation of phospholipase C which hydrolyzes phosphoinositide (PI) to form inositol trisphosphate and diacylglycerol; these two products then act synergistically on protein kinase C to evoke subsequent physiological responses. In smooth muscle, epinephrine and 5-hydroxytryptamine (5-HT) are known to cause muscle contraction through activation of α_1 and 5-HT_2 receptors respectively. We have previously demonstrated for the first time that 5-HT_2 receptors in the rat aorta are linked to PI-specific phospholipase C. Moreover, the 5-HT-induced contraction of aorta is a complicated phenomenon involving not only the activation of phospholipase C but also the voltage sensitive calcium channel. Phorbol esters can induced the tonic component of contraction presumably through activation of protein kinase C. In the cell free system of rat aorta, we have identified the protein kinase C substrates as the myosin light chain (20 k daltons) and another protein with 92.5 k daltons.

In the present study, we have carried out detailed studies of the 5-HT-induced contraction and PI turnover in the rat aorta. We found that the 5-HT-induced contraction and PI hydrolysis were highly correlated with an EC_{50}=10±3 μM for both events (r=0.95, $p < 0.01$) Moreover, the inhibitory potency of a variety of 5-HT_2 antagonists (metergoline, pizotifen, ketanserin, amitryptiline and haloperidol) was highly correlated with binding to the rat brain 5-HT_2 receptor sites (r=0.90; $p < 0.05$). Furthermore, the tumor-promoting phorbol ester, phrobol dibutyrate (PDB) inhibited the 5-HT-induced PI turnover at low nM concentrations without affecting the basal phospholipase C activity. The biologically inactive substance, 4-α-phorbol, was ineffective in affecting 5-HT-induced PI activation. We also found that pretreatment of rat aorta rings with PDB at concentrations which desensitized 5-HT-induced PI turnover also attenuated the maximal tone of contraction induced by 5-HT, especially when a calcium channel blocker nitrendipine was present. These results suggest a feedback regulation of receptor-mediated stimulation of phospholipase C through activation of protein kinase C and phosphorylation of some regulatory proteins.

These studies have provided detailed mechanisms for the 5-HT-induced contraction as well as PI turnover and has assigned a physiological role for the receptor-mediated activation of phospholipase C and protein kinase C. It is conceivable that norepinephrine-mediated aorta contraction is mediated by a similar mechanism. These results also predict that the phospholipase C or protein kinase C inhibitors might be potent vasodilators of great potential for use in critically ill patients. One proposed future project is to prepare primary culture of smooth muscle cells of rat aorta to identify the physiological substrate proteins for protein kinase C following 5-HT_2 and/or norepinephrine receptor activation and to correlate this protein phosphorylation with the contraction of smooth muscle cells induced by 5-HT.

Publications:

Roth, B.L., Nakaki, T., Chuang, D.-M. and Costa, E.: Characterization of 5-HT_2 receptors-coupled to phospholipase C in rat aorta: Modulation of phosphoinositide turnover by phorbol ester. J. Pharmacol. Exp. Ther., 238:480-485, 1986.

Nakaki, T., Wise, B.C. and Chuang, D.-M. Substrates for protein kinase C in a cell-free preparation of rat aorta smooth muscles. Life Science, In press

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02298-02 SMRP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptor Regulation in Cultured Cerebellum Granule Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Xing-Zu Zhu	Visiting Fellow	LPP-NIMH
Ora Dillon-Carter	Chemist	LPP-NIMH
Jian Xu	Visiting Fellow	LPP-NIMH
De-Maw Chuang	Group Chief	LPP-NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on Receptor Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary culture of cerebellar granular cells was prepared fron 8-day old Sprague Dawley rats. We found that these cells possess adrenergic α_1, histaminergic H_1, 5-HT_2 and muscarinic cholinergic receptors linked to phospholipase C. The response of muscarinic receptors stimulated by carbachol was particurlaly robust; this receptor agonist increased by about 30-fold the hydrolysis of phosphoinositide hydrolysis measured by the accumulation of inositol monophosphate (IP_1) in the presence of lithium. This muscarinic receptor response can be desensitized by pretreatment with carbachol or oxotremorine, another muscarinic receptor agonist. This receptor desensitization can be dissected into fast and slow components. The fast component is complete within one hr of stimulation and appears to involve receptor uncoupling. The slow component involves receptor loss assessed by using ^{3}H-QNB and ^{3}H-N-methyl-scopolamine (NMS) as the receptor ligand. The responses of adrenergic α_1, histaminergic H_1 and 5-HT_2 receptors can also be desensitized by stimulation with their respective agonist. However, no heterologous desensitization was found when these receptors were stimulated with their selective agonist for as long as 18 hrs. Cerebellar granular cells also express $GABA_B$ receptor which is linked to inhibition of adenylate cyclase. We found that stimulation of these $GABA_B$ receptors leads to inhibition of voltage sensitive calcium uptake. Moreover, inhibition of this calcium uptake appears to result in attenuation of the release of preloaded D-aspartate from granule cells. These results suggest that voltage sensitive calcium channel rather than the receptor-coupled phospholipase C system plays a major role in regulating the release of the excitatory neurotransmitter from granule cells.

GPO 914-918

Cerebellar granule cells, which are the most numerous and the only known excitatory neurons in the cerebellar cortex, can be dissociated from the tissue of postnatal rats. These primary cultured cells differentiate in vitro to synthesize and release the excitatory transmitter, glutamate. We have attempted to characterize neurotransmitter receptors present in cultured cerebellar granule cells and to decipher the signal transduction systems mediated by these receptors.

For the measurement of receptor-coupled phosphoinositide hydrolysis catalyzed by phospholipase C, granule cells were prelabeled with ^{3}H-inositol and the hydrolysis of labeled phosphoinositide was expressed as the accumulation of ^{3}H-inositol monophosphate (^{3}H-IP$_1$) in the presence of lithium. We found that addition of 5-HT, NE and histamine caused a 2 - 5 fold increase in the accumulation of ^{3}H-IP$_1$. Based on sensitivity to receptor antagonists, we concluded that the effects of 5-HT, NE and histamine are mediated by 5-HT$_2$, adrenergic α_1 and histamine H$_1$ receptors, respectively. Carbachol, a muscarinic cholinergic receptor agonist, caused a robust (30-fold) increase in ^{3}H-IP$_1$ accumulation. The carbachol response was blocked by pirenzepine, a putative muscarinic M$_1$ receptor antagonist, and atropine, a nonselective muscarinic receptor antagonist. The carbachol effect was attenuated by biologically active phorbol ester, a tumor promoting agent, but was unaffected by pertussis toxin. Moreover, carbachol-induced ^{3}H-IP$_1$ accumulation were desensitized by pretreatment with carbachol or oxotremorine, a partial agonist for muscarinic receptor mediated phosphoinositide turnover. The carbachol-induced desensitization could be dissected into fast and slow components. The fast component had a t½ of about 15 min and was virtually complete within one hr of preexposure. During this phase there was no significant loss of binding of ^{3}H-QNB, a membrane permeable ligand and ^{3}H-N-methyl-scopolamine, (NMS), a membrane impermeable ligand, to intact granule cells. In contast, the slow phase of desensitization involves a steady and gradual decline from about 4 to 18 hrs of carbachol-induced ^{3}H-IP$_1$ accumulation and loss of ^{3}H-QNB and ^{3}H-NMS binding to intact cells. Exposure of granule cells to 5-HT, NE and histamine also induced a desensitization of their respective receptor-mediated increase of phosphoinositide turnover. However, no heterologous or cross desensitization to other receptor response was found when cells were exposed to a receptor agonist for as long as 18 hrs. Thus, cerebellar granular cells provide an usual system for studying the regulation of receptor-coupled phospholipase C and its role in the modulation of neuronal function.

Cerebellar granular cells also possess GABA$_B$ receptors coupled to inhibitory adenylate cyclase. We found that activation of GABA$_B$ receptors with (-) baclofen or GABA resulted in a dose dependent inhibition of depolarization (i.e. high K^+) induced $^{45}Ca^{2+}$ uptake into granule cells. Concomittant with this inhibition, we found that the voltage-dependent release of preloaded ^{3}H-D-asparate from granule cells was attenuated. Various biochemical and pharmacological parameters suggest that inhibition of $^{45}Ca^{2+}$ uptake and attenuation of ^{3}H-D-aspartate release are related. Moreover, a voltage sensitive calcium channel blocker, nimodipine, inhibits the K^+-induced release of ^{3}H-D-aspartate in a dose dependent manner. On the contrary, 5-HT, NE, histamine and carbachol did not affect the release of ^{3}H-D-aspartate under conditions in which the activity of phospholipase C was maximally activated. Activators of protein kinase C such as phorbol esters also did not change the K^+-induced release. These data strongly suggest that voltage sensitive calcium channel rather than the phospholipase C system plays a major role in modulating the release of excitatory neurotransmitter from granule cells. Moreover, GABA$_B$ receptor modulates the release of ^{3}H-D-aspartate by inhibition of voltage sensitive calcium channel.

Project Description: Z01 MH 02298-02 SMRP

The present study has provided novel information regarding the regulation and some functional roles of neurotransmitter receptors present in cerebellar granule cells. The bulk of synaptic inputs from spinal cords and brain stem nuclei reaches cerebellum through mossy fibers which innervate granule cells. Granule cells in turn, pass the information through parallel fibers to neurons (such as purkinjie cells) located at the molecular layer of cerebellum cortex. Granule cells are the only cerebellar neuron that utilizes (-) glutamate as the excitatory transmitter. The present study has provided evidence for the functional role of $GABA_B$ receptors of granule cells in modulating the transmitter release. This finding should increase our understanding of mechanisms involved in maintaining a functional equilibrium between excitatory and inhibitory neurotransmissions in the CNS and may further provide a new therapeutic basis for treatment of some mental or neurological diseases resulting from a loss of functional balance of the excitatory pathway. Our major course of future investigations is to address the role of receptor-coupled activation of phospholipase C in the neurophysiological function of cerebellar granular cell neurons. The detailed mechanisms involved in the coupling of neurotransmitter receptors to phospholipase C will also be fully investigated.

Publications:

(1) Xu, J. and Chuang, D.-M. Sertonergic, adrenergic and histaminergic receptors coupled to phospholipase C in cultured cerebellar granule cells of rats. Biochem. Pharmacol. (in press).

(2) Xu, J. and Chuang, D.-M. Muscarinic acetylcholine receptor-mediated phosphoinositide turnover in cultured cerebellar granule cells: desensitization by receptor agonists. J. Pharmacol. Exp. Ther. (in press).

(3) Zhu, X.-Z. and Chuang, D.-M. Modulation of calcium uptake and D-aspartate release by $GABA_B$ receptors in cultured cerebellar granule cells. Eur. J. Pharmacol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02299-02 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Receptor-Mediated Phosphoinositide Turnover

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

De-Maw Chuang	Group Chief	LPP-NIMH
Ora Dillon-Carter	Chemist	LPP-NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on Receptor Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided)*

This investigation aims at the role and regulation of receptor-coupled phospholipase C. We found that in a clonal neurotumor hybrid NCB-20, these cells expresses a variety of receptors of neurotransmitters and neuromodulators that are coupled to phosphoinositide (PI) hydrolysis. Carbachol, a muscarinic cholinergic receptor agonist markedly increased the accumulation of inositol monophosphate (IP_1) in the presence of lithium (Li^+). This increase was time and dose dependent. The formations of inositol bisphosphate and trisphosphate were also increased by this agonist but to a lesser extent and with a faster time course. Antagonist specificity suggests that this effect is mediated by the $M_{1,4,5}$ cholinergic receptors. This activation was associated with a rapid increase in the efflux of ^{45}Ca from cells. NCB-20 cells also contain histamine-sensitive PI turnover system. Antagonist effect indicated that this is a histamine H_1 receptor-mediated response. The neuropeptide, bradykinin, also caused a robust (more than 10-fold) increase of ^3H-IP_1 accumulation. The effects of bradykinin, histamine and carbachol were additive to each other. The carbachol-induced ^3H-IP_1 accumulation was completely inhibited by veratridine at concentrations that inhibit the voltage-sensitive sodium channel. The basal accumulation was increased 2-fold by veratridine, while the activities increased by histamine and bradykinin were unaffected. The responses of both bradykinin and carbachol were desensitized by preexposure to bradykinin and carbachol, respectively. Biologically active phorbol esters markedly attenuated the bradykinin and carbachol-receptor mediated responses and significantly inhibited the basal phospholipase C activity, suggesting that either phospholipase C or some phospholipase C regulatory proteins are substrates of phorbol ester-activated protein kinase C. Pertussis toxin only attenuated in part the carbachol and bradykinin-induced PI turnover. The concentration of lithium required to maximally increase the receptor-activated ^3H-IP_1 accumulation in cultured NCB-20 cells was about 60-80 mM which was about 10-time greater than those found in brain slices and other neurotumor cell lines such as NG 108-15 neurohybrid. This unusual requirement of high lithium may suggest that NCB-20 cells express a novel type of inositol-1-phosphatase and might lead to some clinical implication for the mechanisms of the therapeutic effect of lithium in the treatment of manic depression.

The receptor-coupled increase of poly-phosphoinoside hydrolysis (PI) turnover is a phenomenon well established in a variety of tissues. This receptor agonist mediated event is due to activation of membrane-bound phospholipase C with subsequent hydrolysis PI to form two key metabolites, diacylglycerol and inositol trisphosphate (IP_3). Both of which may serve as a second messager to trigger the signal cascade. Diacylglycerol stimulates a calcium and phospholipid-dependent protein kinase C, where as IP_3 appears to act by mobilizing intracellular calcium. The brain is particularly enriched in phospholipase and protein kinase C. Hence this receptor-coupled generation of second messengers is likely to play a fundamental role in neuronal function.

The study of receptor-mediated PI hydrolysis in brain slices has been complicated by the presence of an extremely heterogenous cell population and sometimes hampered by a relatively small signal of activation. In this report, we used the NCB-20 cell which is a stable neurotumor line derived from fusion between mouse neuroblastoma and Chinese hamster 18-day embryonic brain cell, to study the mechanisms of neurotransmitter receptors-coupled PI hydrolysis. NCB-20 cells were grown to confluency and then incubated overnight with ^{3}H-myo-inositol to label the endogenous inositol phosphoinositide. The turnover of PI was measured by the accumulation of ^{3}H-inositol monophosphate (IP_1) in the presence of lithium, which has been shown previously to inhibit the inositol-1-phosphatase activity.

Additions of carbachol, histamine and bradykinin were found to increase the accumulation of ^{3}H-IP_1 with a maximal activation of approximately 5-, 3- and 10- fold, respectively. The EC_{50} of carbachol was about 50-70 μM and the effect was blocked by atropine, a nonselective muscarinic cholinergic receptor antagonist,and pirenzepine, a selective muscarinic M_1 receptor antagonist, with a Ki of 0.5 and 25 nM, respectively. Bethanecol and pilocarpine were partial agonists in promoting the response, whereas oxotremorine, McN-A-343 and AHR-602 were virtually inactive. The formations of inositol bis-and trisphosphates were also increased but these increases were faster in time course and smaller in magnitude when compared with the parameters of IP_1. The histamine response (EC50=50uM was blocked by two histamine H_1 receptor antagonists, triprotiline and phenhydramine but was unaffected by a histamine H_2 receptor antagonist, cimetidine. The effects of bradykinin, histamine and carbachol were additive to each other at concentrations that displayed maximal activation of PI turnover. The carbachol-induced ^{3}H-IP_1 accumulation was completely inhibited by veratridine at concentrations that inhibit the voltage sensitive sodium channel, while the activities increased by histamine and bradykinin were unaffected. The basal activity was increased by about two-fold by veratridine treatment. Since activation of muscarini cholinergic receptors in neuroblastoma has been shown to increase the sodium influx, it is conceivable that the veratridine effect represents a feedback regulation of the muscarinic cholinergic response by opening a voltage sensitive sodium channel.

The responses of both bradykinin and carbachol were desensitized by preexposure of NCB-20 cells to bradykinin and carbachol, respectively. This desensitization involved a loss of the maximal extent of receptor-activated PI turnover and was dependent on the time of preexposure to their respective receptor agonist. Biologically active phorbol ester such as phorbol myristate acetate (PMA) and phorbol dibutyrate (PDB) markedly attenuated the carbachol and bradykinin-mediated response with an IC_{50} of about 20 nM. The basal activity of phospholipase C was also significantly inhibited by these phorbol esters. These data imply that either phospholipase C or some regulatory protein(s) for this enzyme may be the substrate for a protein kinase C which is activated by phorbol esters. We also found that the concentration of lithium required to maximally increase the receptor-activated IP_1

accumulation in NCB-20 cells was about 60-80 mM which was about 10-time greater than those found in brain slices and other neurotumor cell lines such as NG108-15 neurohybrid. This requirement of high concentration of lithium for eliciting IP_1 accumulation may suggest that NCB-20 cells express an unusual form of inositol-1-phosphatase which is more resistant to inhibition by lithium of its enzymatic activity.

The CNS is particularly active in metabolizing the phosphoinositide and is very enriched in protein kinase C. It is therefore likely that the receptor-mediated activation of the phospholipase C system plays a crucial in modulating the neurotransmission. In support of this context, Higashida and coworkers have shown that in NG108-15 cells, mobilization of intracellular calcium by IP_3 generated from bradykinin-activated hydrolysis of PI opens a calcium dependent potassium current, while activation of protein kinase C by phorbol esters closes a voltage-sensitive potassium channel (Proc. Natl. Acad. Sci. USA, 83, 942-946, 1986). In the present study, we showed that NCB-20 cells express muscarinic cholinergic, histaminergic H_1 and and bradykinin receptors coupled to phospholipase C. These receptor responses are regulated by phorbol esters and pertussix toxin and can be desensitized by prestimulation with their receptor agonist. These novel information has increased our understanding of the regulatory mechanisms of neurotransmission mediated by receptor-activated PI turnover. The presence of an unusual form of inositol-1-phosphatase in NCB-20 cells may be of great importance in understanding the mechanisms of action of lithium. Since inositol-1-phosphatase is a possible site of action of lithium in the treatment of manic depression, our finding that high lithium is required to inhibit the activity of this phosphatase may have implication for some clinical cases in which bipolar depressive patients are not beneficial from treatment with lithium. The major courses of future studies are to further investigate the mechanisms involved in the regulation of receptor-coupled phosphoinositide hydrolysis and to explore its role in the neurophysiological function.

Publication:

Chuang, D.-M., Carbachol-induced accumulation of inositol-1-phosphate in neurohybridoma NCB-20 cells: Effects of lithium and phorbol esters. Biochem. Biophys. Res. Commun. 136: 622-629, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02300-02 SMRP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Neurotransmitter Receptors by Cell Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Xing-Zu Zhu	Visiting Fellow	LPP-NIMH
De-Maw Chuang	Group Chief	LPP-NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

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Receptor Pharmacology Group

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS.	PROFESSIONAL	OTHER
1.2	1.2	None

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The understanding of the molecular mechanisms of the differentiation of neurons during development is of fundamental importance in neuroscience. We have used two neuronal hybridomas, NCB-20, and NG108-15 cells, to study the receptor regulation induced by two differentiation promoters, dibutyryl cAMP (Bt_2cAMP) and butyrate. Exposure of cells to Bt_2cAMP caused a time and dose dependent decrease in the binding to muscarinic acetylcholine, adrenergic α_2 and opioid δ receptors. The loss of muscarinic cholinergic receptor site assessed by using ^{3}H-QNB as the binding ligand was about 40% of the control; this decrease was associated with an attenuation of the carbachol-induced phosphoinositide hydrolysis. The maximal decreases of adrenergic α_2 and opioid δ receptor binding were about 80% when ^{3}H-clonidine and ^{3}H-D-Ala-D-Leu-enkephalin (DADLE) were used as their respective ligands. In contrast, butyrate induced a time and dose dependent increase in the opioid receptor binding sites. The maximal increase was about 300% of the control when 1 mM of butyrate was added to the culture for 72 hrs. The up-regulation of opioid receptor resulted in an increased potency of DADLE in inhibiting adenylate cyclase activity. Similar butyrate treatment up-regulated muscarinic receptors by 100% and increased carbachol-induced phosphoinositide by about 200%. Long term butyrate treatment of NG108-15 cells did not change muscarinic and opioid receptor binding but induced a 100% increase of the density of α_2-adrenergic receptors. Butyrate treatment induced cell morphological changes which were distinct from those induced by Bt_2cAMP. These results suggest that these two differentiation agents can either up-regulate or down-regulate neurotransmitter receptor proteins. Moreover, up-regulation of neurotransmitter receptors induced by butyrate may involve concerted interactions of genetic factors derived from both parents of these two neurohybrids.

PHS 6040 (Rev. 1/84) GPO 914-918

The study of molecular mechanisms of neuronal differentiation in the brain has been hampered by the problems arising from the heterogeneity of cell population and the complexity of their interactions. In fact, not only did the glias out number the neuron by 10 to 1 but also the neurons are extremely heterogeneous in nature. These complications may be minimized by the use of clonal neurohybrid cell lines such as NCB-20 and NG108-15 cells. NCB-20 and NG108-15 cells express many properties characteristic for neurons. For example, NCB-20 cells contain choline acetytransferase, 5-HT_1 receptor-linked adenylate cyclase, α_2-adrenergic, δ -opioid and D_2-dopaminergic receptors. Both cell lines possess excitable membranes and form synapses with myotubes. It has also been shown recently that NCB-20 cells express muscarinic cholinergic receptor linked to phosphoinositide (PI) turnover (Chuang, Biochem. Biophys. Res. Commun. 136:622-629, 1986). In this report we have used the dibutyryl cAMP (Bt_2cAMP) and butyrate-induced differentiation to study their effects on the properties of receptors for muscarinic acetylcholine, adrenergic α_2 and opioid δ in these two cell lines.

Long term treatment of NCB-20 cells with sodium buyrate resulted in a marked increase in the binding of ^{3}H-D-Ala^2, D-Leu^5 enkephalin (^{3}H-DADLE) to delta-opioid receptors. The butyrate-induced increase was concentration and time dependent with an EC_{50} of about 480 μM and a maximal effect detected after 3-day treatment. At saturating concentration of butyrate (1 mM) the increase was 3 - 4 fold of the untreated control. Scatchard analysis revealed that the butyrate effect was due to an increase in the density of the opioid receptor binding sites. Butyrate also induced a smaller (110%) increase in muscarinic cholinergic receptor binding assessed by using ^{3}H-quinuclidinyl benzilate (^{3}H-QNB), while alpha$_2$-adrenergic receptor binding assessed by using ^{3}H-clonidine was virtually unaffected. The butyrate-induced opioid receptor binding was totally abolished by the presence of cycloheximide, suggesting that the butyrate effect involves synthesis of the receptor protein. Butyrate treatment did not affect basal and prostaglandin E_1 (PGE_1)-stimulated cAMP levels but caused a 3 - 4 fold decrease in the IC_{50} of DADLE for attenuating these cAMP levels and approximately 25% increase in the maximal extent of attenuation. In contrast to the butyrate effect, long term treatment of NCB-20 cells with 1 mM dibutyryl cAMP (Bt_2cAMP) induced an 80% decrease in the specific binding of ^{3}H-DADLE and ^{3}H-clonidine and a 57% loss of ^{3}H-QNB specific binding. The loss of ^{3}H-QNB binding was associated with a 35% decrease of carbachol-induced phosphoinositide breakdown, while up-regulation of ^{3}H-QNB binding sites resulted in about 200% increase of carbachol-induced phosphoinositide hydrolysis. The differential regulation by butyrate and Bt_2cAMP suggests that the butyrate effect is mediated by a mechanism independent of intracellular cAMP. Butyrate and Bt_2cAMP also induced distinct morphological changes in NCB-20 cells. While Bt_2cAMP markedly increased the formation of neurite-like processes, butyrate only induced apparent clumping the cells grown in monolayer culture.

In contrast to the effect of butyrate on NCB-20 cells, long term butyrate treatment of NG108-15 cells failed to increase the numbers of delta-opioid receptors and muscarinic cholinergic receptors but induced a 100% increase in α_2-adrenergic receptors binding assessed by using ^{3}H-clonidine as the receptor ligand. This increase in ^{3}H-clonidine in NG108 cells binding were blocked by the co-presence of cycloheximide. These results indicate that the induction pattern by butyrate of neurotransmitter receptors in NCB-20 and NG108-15 cells are dramatically different. Moreover, while long term Bt_2cAMP treatment of NCB-20 cells down-regulates opioid, muscarinic cholinergic and α_2-adrenergic receptors, such treatment of NG108 cells had no effect on these three types of receptor binding characteristics. Thus, up-and down-regulations of neurotransmitter receptors in these two

neurohybird cell lines require concerted interactions between genetic factors derived from both neurohybrid parents. These cell lines may provide excellent model systems for studying the regulatory mechanisms of gene expression.

The significance of this study on biochemical research is that differentiation of a neuron induced by increasing cAMP level is associated with down-regulation of certain neurotransmitter receptors, while butyrate which induced different morphological differentiation can up-regulate a selective class of receptors, depending on the type of cell lines studied. These up- and down-regulations of receptors appear to result in alterations in the effector responses coupled to these receptors. These results also imply that differentiation involves not only the induction of new messenger RNA but also the disappearance of old messenger RNA. This information has increased our understanding of molecular basis of some neuropathological diseases associated with abnormalities in neuronal differentiation and may provide a new therapeutical basis for these illnesses.

The proposed courses of this project are (1) to define the molecular genetic events involved in butyrate-induced up-regulation of neurotransmitter receptors in NCB-20 and NG-108 cell lines and other pertinent cell cultures such as primary culture of cerebellar granule cell neurons and (2) to study the effects of differentiation agents including butyrate in vivo systems.

Publications:

1. Zhu, X.-Z. and Chuang, D.-M. Differential regulation by butyrate and dibutyryl cAMP of delta-opioid, alpha$_2$-adrenergic and muscarinic cholinergic receptors in NCB-20 cells. J. Neurochem, (in press).

2. Zhu, X.-Z. and Chuang, D.-M. Comparision of the butyrate effects on neurotransmitter receptors in neurohybrid NG108-15 and NCB-20 cells. Life Sci. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02301-02 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Functional Role of Adrenal NPY

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H.-Y. T. Yang	Section Chief	LPP-NIMH
E. A. Majane	Chemist	LPP-NIMH

COOPERATING UNITS *(if any)*

Terry D. Hexum, PH.D., Associate Prof., Dept. of Pharmacology, University of Nebraska, Medical Center, Omaha, Nebraska

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuropeptide

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High concentrations of neuropeptide Y have been found in many norepinephrine containing chromaffin cells in adrenal glands of various species. Adrenal gland seems to provide an interesting model system in which to investigate the functional role of NPY because evidence is accumulating that NPY may have modulatory effect on catecholamine. Previously, we have found that NPY can be released from bovine adrenal by acetylcholine. In the present study, the cholinergic receptor mediated secretion was further characterized by using retrogradedly perfused bovine adrenal glands. Infusion of acetylcholine produced a dose dependent increase in NPY secretion. Hexamethonium antagonized this NPY release whereas atropine did not. The role of the nicotinic cholinergic receptor in this process was further established by examining the effect of 1,1-dimethyl-4-phenlpiperazinium (DMPP). Infusion of DMPP also stimulated the NPY secretion. Biochemical analysis by HPLC revealed that four NPY immunoreactive peptides were secreted and the major immunoreactivity was identified to be authentic NPY. This release pattern of NPY is similar to that of catecholamines, thus it may be suggested that NPY is co-released with catecholamines by exocytosis via stimulation of nicotinic receptor. Previously, we have found that NPY levels in rat adrenal glands increase markedly with age during maturation and furthermore, an additional NPY-like peptide is detected in adrenal glands of older rats but not in that of younger rats. The nature of this NPY-like peptide is presently unclear; however, further characterization of this NPY-like peptide indicates that it is not an oxidized form of NPY, PYY or a degraded fragment of NPY. Furthermore, this NPY-like peptide is present in splanchnic nerves and also in chromaffin cells and can be secreted upon stimulation of cholinergic receptor. Highly purified NPY-like material has been prepared from bovine adrenal glands and we are planning to examine the biological activity of this new peptide. It is known that NPY exerts direct vasopressor effect and potentiates catecholamine induced vasoconstriction; furthermore, NPY can be co-released with catecholamines from adrenal glands thus NPY may contribute to the cardiovascular effects seen upon adrenal activation.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description: Z01 MH 02301-02 SMRP

High concentrations of neuropeptide Y (NPY) have been found in many norepinephrine containing chromaffin cells in adrenal glands of various species. Evidence is accumulating that NPY may have modulatory effect on catecholamine, thus, adrenal glands seem to provide an interesting model system in which to study the functional role of NPY. Previously, we have demonstrated that similar to catecholamines NPY can be released from bovine adrenal glands by stimulation with acetylcholine. In this study using retrogradedly perfused bovine adrenal glands and radioimmunoassays, the NPY secretion was further characterized and compared with the enkephalin secretion. Infusion of either acetylcholine or 1,1-dimethyl-4-phenylpiperazinium (DMPP) into the bovine adrenal gland produced a significant increase in the perfusate concentration of NPY and met^5-enkephalin immunoreactivities. The amounts of peptides released are dependent on the dose of acetylcholine or DMPP and an increase of greater than 250% over basal release was observed. Administration of the cholinergic recepter antagonists, atropin and hexamethonium prior to the infusion of acetylcholine or DMPP was carried out to assess the receptor subtypes. Hexamethonium antagonized the secretion of NPY and met^5-enkephalin immunoreactivities implicating a role for the nicotinic receptor in the secretion of these two peptides. Atropin did not affect the release of NPY induced by acetylcholine but unexpectedly inhibited the release of NPY induced by DMPP. The reason for this unexpected observation is unclear at present. Given the fact that NPY is stored in chromaffin granules and its release pattern is similar to that of the catecholamines, it can be concluded that NPY and catecholamine are co-released by exocytocis.

Previously, we found that rat adrenal NPY increased markedly with age during maturation and, furthermore, an additional NPY-like peptide was detected in the adrenal gland of older rat. In the present study, this NPY-like peptide is further characterized and its purification is in progress. The exact nature of this peptide is still unclear, however, the characterization of this NPY-like peptide indicated that it is not an oxidized form of NPY, PYY or a degraded fragment of NPY. Interestingly, this NPY-like peptide is present in significant quantity in adrenal glands of older rats but not in that of younger rats. Furthermore, this NPY-like peptide is detected in chromaffin cells of bovine adrenal glands and splanchnic nerves of pig adrenal, and can be secreted from bovine adrenal glands when stimulated by acetylcholine. By isolation of chromaffin granules followed with successive steps of column chromatography, a highly purified NPY-like material has been prepared and we will attempt to determine the biological activity of this new peptide.

Significance to Biomedical Research:

The results of this study suggest that NPY can be co-released with catecholamines from the adrenal gland. It is known that NPY exert direct vasopressor effect and enhances catecholamine induced vasoconstriction, thus it is possible that NPY contributes to the cardiovascular effects seen upon adrenal activation.

Proposed Course of Study:

We plan to purify the new NPY-like peptide to homogeneity and then characterize this peptide chemically and also biologically.

Publications:

Higuchi, H., Costa, E. and Yang, H.-Y. T.: Neuropeptide Y inhibits the nicotine mediated release of catecholamines from bovine adrenal chromaffin cells. J. Pharmacol. Exp. Ther. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02378-01 SMRP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Histochemical Localization of Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 Immunoreactivity in Mammalian CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

T. Salminen	Visiting Fellow	LPP-NIMH
E.A. Majane	Chemist	LPP-NIMH
H.-Y.T. Yang	Pharmacologist	LPP-NIMH

COOPERATING UNITS *(if any)*

C.-H. Lee, Naval Medical Research Institution, Bethesda, Maryland

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Section on Neuropeptides

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The tetrapeptide phe-met-arg-phe-NH_2 (FMRF-NH_2) was originally isolated from macrocallista nimbosa clam and subsequently existence of FMRF-NH_2-like peptides in mammalian CNS was demonstrated by the antiserum raised against FMRF-NH_2. Recently, two of these FMRF-NH_2-like peptides were isolated from bovine brain and chemically characterized. In this study, monoclonal antibody against one of these FMRF-NH_2-like peptides of mammalian origin, phe-leu-phe-gln-pro-gln-arg-phe-NH_2 (F-8-F-NH_2), was generated and will be used to study the distribution of F-8-F-NH_2 in detail by immunohistochemical technique. By using F-8-F-NH_2 conjugated to hemocyanin as antigen, three murine monoclonal antibodies of IgG isotype were produced through cell fusion and cloning. The specificity study indicated that those antibodies cross-reacted with peptides having arg-phe-NH_2 at their c-termini but showed no cross-reaction with NPY, PYY or met^5-enk-arg^{6}phe^7. The result indicates that these monoclonal antiodies will enable us to carry out the immunohistochemical localization of F-8-F-NH_2 or FMRF-NH_2 immunoreactivity and compare this distribution with the distribution of NPY. Some immunohistochemical studies using polyclonal antiserum indicate that it is difficult to distinguish between mammalian FMRF-NH_2-like immunoreactivity from NPY due to cross-reactions of antisera. Extracts from spinal cord was analyzed by high pressure liquid chromatography coupled with radioimmunoassay using the monoclonal antibody. The major immunoreactivity was identified as F-8-F-NH_2 and NPY was not recognized by the antibody, thus the specificity of the monoclonal antibody was further confirmed. In the periaqueductal gray area, the preliminary study using the monoclonal antibody showed numerous immunoreactive nerve fibers around blood vessels and capillaries. Network of F-8-F-NH_2 positive nerve fibers was found surrounding the cerebral aqueduct and running in the epithelium. We plan to extend this immunohistochemical study to other regions of CNS and hope to provide a frame work for further understanding of possible function of F-8-F-NH_2 or mammalian FMRF-NH_2-like peptides.

GPO 914-918

Recently, a novel peptide, phe-leu-phe-gln-pro-gln-arg-phe-NH_2 (F-8-F-NH_2) originally detected by antiserum raised against phe-met-arg-phe-NH_2 (FMRF-NH_2), was isolated from bovine brain and found to modulate morphine analgesia.[2] Using the antiserum raised in rabbits to this peptide, it was shown to distribute unevenly in CNS with highest concentrations in dorsal spinal cord and periaqueductal gray, areas important in opiate-mediated pain perception. These two areas are known to also contain high concentration of neuropeptide Y (NPY). Some immunohistochemical studies indicated that FMRF-NH_2-like and NPY immunoreactivities are both stored in the same neurons in spinal cord. However, it is not clear whether it is possible to differentiate NPY from FMRF-NH_2-like peptides by immunohistochemical technique using polyclonal antisera which often lack necessary specificities. Therefore, in this study, monoclonal antibodies were generated in an attempt to select an antibody capable of differentiating NPY from F-8-F-NH_2 which is one of the mammalian FMRF-NH_2-like peptides. By using F-8-F-NH_2 conjugated to hemocyanin as antigen, three murine monoclonal antibodies of IgG isotype to F-8-F-NH_2 were obtained through cell fusion and cloning. Specificity of these antibodies was examined by the inhibition of [^{125}I]-tyr-leu-phe-gln-pro-gln-arg-phe-NH_2 binding by various peptides. The antisera cross-reacted with peptides having arg-phe-NH_2 at their c-termini but showed no cross-reaction with NPY and PYY, peptides with arg-tyr-NH_2 at their c-termini, or met^5-enk-arg^6-phe^7. This result suggests that these monoclonal antibodies recognize the c-terminal portion of F-8-F-NH_2 and that arg-phe-NH_2 is required for the immunoreactivity. It also indicates that these monoclonal antibodies are capable of differentiating - arg-phe-NH_2, the c-terminal dipeptide amide of FMRF-NH_2-like peptides from - arg-tyr-NH_2, the c-terminal dipeptide amide of NPY. Extracts from spinal cords of bovine and rat were analyzed by high pressure liquid chromatography coupled with radioimmuoassay using the monoclonal antibodies. The monoclonal antibody recognized the FMRF-NH_2-like peptides of both species but not NPY. The specificity of these monoclonal antibodies is thus further confirmed and will be useful in differentiating the distribution of F-8-F-NH_2 or mammalian FMRF-NH_2-like peptides from that of NPY.

F-8-F-NH_2 was found to be highly localized in periaqueductal gray by the radioimmunoassay. The preliminary immunohistochemical study with the monoclonal antibody showed numerous immunoreactive nerve fibers around blood vessels and capillaries in periaqueductal gray area. Network of F-8-F-NH_2 positive nerve fibers was found surrounding the cerebral aqueduct and running in the epithelium. We are currently examining the specificity of these immunostaining by pre-abosorption of the antibody with F-8-F-NH_2 and also NPY.

Significance to the Biomedical Research:

Several lines of evidence suggest that FMRF-NH_2-like peptides including F-8-F-NH_2 may function as an endogenous antiopiate peptide.[2] With the availability of the specific monoclonal antibody, we hope to be able to study the distribution of F-8-F-NH_2 exclusive of NPY by immunohistochemical technique and to gain a better understanding of the possible role of F-8-F-NH_2 which is one of the FMRF-NH_2-like peptides of mammalian origin.

Proposed Course of Study:

To study the distribution of F-8-F-NH_2 in rat CNS with immunohistochemical technique using monoclonal antibody and also to distinguish the distribution of F-8-F-NH_2 from that of NPY.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02316-02 CBDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

"Teaching the Wisconsin Card Sort of Schizophrenic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Clinical brain Disorders Branch

Dr. Daniel Weinberger, Chief, Section on Clinical Brain Disorders Branch NPB, IRP, NIMH; Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH; Dr. Marvin Podd, O'Malley Division, Saint Elizabeths Hospital

COOPERATING UNITS *(if any)*

O'Malley Division, Saint Elizabeths Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg., Saint Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [X] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Project has been completed.

PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02351-01 CBDB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathology of Selected Central Nervous System Degenerative Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. M.F. Casanova, Neurologist and Neuropathologist, NIMH

Dr. D. Price, Director Neuropathology Laboratory, Johns Hopkins University, Dr. G. Moore, Assistant Professor of Pathology, Johns Hopkins University, Dr. L. Cork, Associate Professor of Veterinary Medicine, Johns Hopkins University, Dr. R. Struble, Instructor of Pathology, Johns Hopkins University, Dr. P. Whitehouse, Associate professor, Case Western University.

COOPERATING UNITS *(if any)*

Clinical Brain Disorders Branch, NIMH; Neuropsychiatry Branch, NIMH.

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section On Neuropathology Studies

INSTITUTE AND LOCATION
NIMH, WAW Bldg., St. Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Selected neuropsychiaric conditions (Alzheimer's Disease (AD), Schizophrenia (SC), Dystonia musculorum deformans (DMD) and Progressive Supranuclear Palsy (PSP) were studied with quantitative anatomical techniques in order to establish clinico-pathological correlates and ellucidate pathogenesis. The structures incriminated by our research were further studied during gestation to discern possible developmental insults which could give rise to these neuropsychiatric conditions.

A. In AD: We described sequestration of tubulin in granulovacuolar degeneration and the involvement of various neuropeptidergic systems in the abnormal neurites surrounding plaques.

B. In DMD: We provided the first report of pathology involving various brainstem structures.

C. In PSP: We described cell loss in a major cholinergic non-motor nuclei in the brainstem.

D. In SC: We reviewed a published report on astrocytosis but could not confirm their findings.

The significance of the project lies in the identification of pathological correlates to these neuropsychiatric conditions as a first step towards the discovery of their etiology and possible therapeutic interventions.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description: Z01 MH 02351-01 CBDB

Objectives: To examine autopsy tissue for abnormal neuronometrics, immunocytochemical and autoradiographic changes in the brains of Alzheimer's Disease (AD), Schizophrenia (SC), Dystonia Musculorum Deformans (DMD) and Progressive Supranuclear Palsy (PSP) patients. The areas to be studied represent possible sites of pathology as suggested by the clinical manifestations of the disease. Hopefully, delineation of pathological changes will provide important clues as to the pathophysiology of the disease and permit eventual investigations of their etiology and possible therapeutic interventions.

Methods Employed: Brains are collected from the D.C. medical examiners and processed accordingly for computer image analysis, immunocytochemistry and/or autoradiography. The emphasis is on quantitative techniques and the combined use of anatomical and neurochemical studies.

1) Computer-assisted image analysis: Deals with problems of quantitative microscopy in the assessment of different neuronal pupulations.

2) Immunocytochemistry: Combining the standard method of the Sternberger's with acrolein as a fixative provides the opportunity to qualitatively describe the presence and distribution of antigen in tissue.

3) Autoradiography: Computer-assisted densitometric analysis of grain density on film is used to define receptor concentrations in specific anatomical areas.

New Findings:

1) In AD: We described sequestration of tubulin in granulovacuolar degeneration and the involvement of various neuropeptidergic systems in the abnormal neurites surrounding plaques. These findings combined with our previous description of phosphorylated neurofilament antigen in neurofibrillary tangles (J Neuropathol Exp Neurol 45: 56-64, 1986) suggest a basic cytoarchitectural disruption in certain large neurons in AD.

2) In DMD: We provided the first pathological correlate for primary (hereditary) forms of dystonia and a possible explanation for both sleep and EEG abnormalities

3) In PSP: We describe cell loss in a major cholinergic non-motor nuclei in the brainstem.

4) In SC: We reviewed a published report regarding astrocytosis in this illness but could not confirm their findings.

5) In development: We confirmed the cholinergic nature of striatal "striosomes" and described similar patterns of innervation during development for adrenergic and dopaminergic receptors.

Significance to Mental Health Research: Delineation of these pathological changes in AD, DMD, PSP, and SC provides important clues as to the pathophysiology of these diseases and will hopefully, permit eventual investigations of their etiology and possible therapeutic interventions.

Proposed Course of Project: We will concentrate most of our research effort in schizophrenia. The use of computer assisted image analysis will allow quantitation of pigments (e.g.: lipofuscin and iron) and provide measurements of disarray or tangledness in the pyramidal cells of the hippocampus. At the same time we will combine anatomical and neurochemical techniques in the study of structures of the limbic system with emphasis on the entorhinal cortex.

Publications

Moore, G.W., Polocsek, R.A., Casanova, M.F., Erozan, Y.S. Hershey, J., Miller, R.E., and Hutchins G.M., : Multilingual respelling rules for an English medical word list. In: MEDINFO 86. R. Salamon, B., Blum and M. Jorgensen (eds), Amsterdam, Elsevier Science Publishers 1987, pp. 1106-1110.

Price, D.L., Altschuler, R.J., Struble, R.G., Casanova, M.F., Cork, L.C., and Murphy D.B.: Sequestration of tubulin in neurons in Alzheimers disease. Brain Res. 385: 305-310, 1986.

Casanova, M.F., Troncoso J.C., and Price D.L.,: Cerebral hemorrhogic infarcts: An autopsy study of 76 cases. Bol. Aeoc. Med. P.R. 79: 7-11, 1987.

Zweig, R.M., Whitehouse, P.J., Casanova, M.F., Walker L.C., Jankel, W.R., and Price, D.L.,: Loss of pedunculopontine neurons in progressive supranuclear palsy. Ann Neurol, in press.

Lowenstein, P.R., Slesinger, P.A., Singer, H.S., Walker L.C., Casanova, M.F., Price, D.L., and Coyle, J.T.,: An autoradiographic study of the development of 3-hemicholinium binding sites in human and baboon basal ganglia: A marker for the sodium dependent high affinity choline uptake system. Dev. Brain Res., in press.

Struble, R.G., Powers, R.E., Casanova, M.F., Brown E.C., Keith, C.A., and Price D.L., : Neuropeptidergic systems in plaques of a Alzheimer's disease, Journal of Neuropathology and Experimental Neurology (in press).

Casanova, M.F., Stevens, J., Bigelow L.,: On the quantitative evaluation of enzyme immunocytochemistry staining in human postmortem material, Biol., Psy. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02352-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prefrontal Cortical Modulation Of Subcortical Dopamine Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. George Jaskiw, Clinical Brain Disorders Branch, NIMH; Dr. Farouk Karoum, Neuropsychiatry Branch, NIMH; Dr. William Freed, Neuropsychiatry Branch, NIMH; Dr. Joel Kleinman, Clinical Brain Disorders Branch, NIMH; Dr. Daniel Weinberger, Clinical Brain Disorders Branch, NIMH.

COOPERATING UNITS *(if any)*

Clinical Brain Disorders Branch, NIMH; Neuropsychiatry Branch, NIMH.

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section On Clinical Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg, St. Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have examined the relationships between catecholamine systems in the prefrontal cortex (PFC) and subcortical areas, notably the nucleus accumbens and corpus striatum. Our work suggests that chemical lesioning of efferents from the prefrontal cortex to subcortical areas does not markedly alter behaviors such as stereotypy and locomotion, believed to be mediated by dopamine systems in the latter. Along with other work, this suggests that dopamine afferents to prefrontal cortex play a unique role in modulating subcortical dopamine systems.

PHS 6040 (Rev 1/84) GPO 914-918

Project Description:

Objectives: The regulation of dopamine turnover in subcortical areas is still poorly understood. Several studies suggest that dopamine in PFC inhibits subcortical dopamine release and turnover, as well as downregulate subcortical dopamine receptors. We set out to elucidate the relationship between PFC and subcortical DA systems.

Methods Employed: Stereotactic neurotoxic lesions were made in the PFC of male rats. After recovery rats were tested for activity and stereotypy in response to dopamine agonist challenges. Rats were subsequently sacrificed and various cerebral regions have been dissected out for analysis of catecholamine metabolites.

Major Past Findings: Our work began in the fall of 1987. We did not report any findings before the current period.

New Findings: Bilateral lesions of PFC using ibotenic acid, a toxin which destroys neuronal bodies while sparing axons of passage, did not significantly affect activity or stereotypy responses induced by apomorphine or amphetamine administered at different times, and at several doses.

Our work suggests that neurons with cell bodies in the PFC do not exert a tonic inhibition on subcortical dopamine turnover, since release of the accumbens and corpus striatum from control of the PFC did not affect behaviors mediated by subcortical DA systems.

Significance to Mental Health Research: A large body of evidence implicates pathophysiology of subcortical dopamine systems in schizophrenia. Current psychopharmacologic approaches almost exclusively aim at blocking dopamine transmission in the subcortical areas. Unfortunately such treatment is only partially effective in some patients, and is associated with unpleasant side-effects (tardive dyskinesia). Elucidation of the control of subcortical dopamine systems may lead to development of more effective and safer drugs for the treatment of schizophrenia.

Proposed Course of Project: Three related approaches are expected to advance this work. 1) the experiment will be repeated using 6-hydroxydopamine after desipramine pretreatment, to replicate findings of other researchers 2) the selective stress response of PFC DA neurons will be examined in controls and rats with PFC lesions 3) rats will be implantated with pumps which chronically deliver small quantities of dopamine agonists into the PFC; behavioral and biochemical testing will be follow.

Publications:

Jaskiw, G.E., Weinberger, D.R.: The prefrontal cortex-accumbens circuit: Who's in Charge? The Behavioral and Brain Sciences (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02353-02 CBDB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cranial Asymmetries And The Reliability Of The International 10-20 System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Principal Investigator: Michael Myslobodsky, M.D., Clinical Brain Disorders Branch, NIMH

Others: Richard Coppola, D.Sc., Clinical Brain Disorders Branch, NIMH, Craig Karson, M.D., Clinical Brain Disorders Branch, NIMH, David Daniel, M.D., Clinical Brain Disorders Branch, NIMH, Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section on Clinical Studies

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	3	0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have found that inconspicuous cranial asymmetry may covary with the lateral distribution of the EEG. Occipital flattening correlated with increased alpha in the subdominant parietal and central leads and increased beta power in caudal dominant leads. Frontal asymmetry ("frontal buldging") correlated with a relative increase of delta over the midline and the dominant parieto-occipital leads, with a generalized increase in alpha power, and with increased beta in dominant parietal and central leads. This study suggests that it might be desirable to verify electrode positions on the head on the basis of CT-MRI findings in all cases where brain laterality is at issue.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: The widely used international 10-20 system adopted for clinical and research electrophysiology does not guarantee an acceptable fit between an electrode and the target brain site. The skull is seldom perfectly symmetrical and the skull landmarks that are used to compute electrode locations are not precisely related to the brain anatomy. However, it has to be shown which normally occurring cranial asymmetries matter; which electrode locations are most hazardous in contributing the EEG imprecision? These two questions were assessed in the present study.

Methods Employed: Craniometric approach used for plagiocephaly was modified for quantification of lateral asymmetry indices of the frontal and occipital quadrants. In several individuals EEG was examined and the values of cranial asymmetry were compared with EEG power in the alpha, beta, theta, and delta frequency bands.

Major Past Findings: Our pilot study showed that slight flattening of the occipital bone on one side or asymmetries of the petrous ridge, that can be easily quantified using CT images, covary in a reliable way with the imbalance of EEG power in the alpha and beta bands in both frontal occipital regions.

New Findings: (1) Asymmetry of the frontal bosses and relative unilateral flattening of the occipital lobe covaried with changes in the delta,alpha, and beta bands, but not in the theta band. Frontal asymmetry ("frontal bulging") correlated with a relative increase of delta-power over the midline and the dominant parieto-occipital leads, generalized and central leads. Occipital flattening was correlated with increased beta power in caudal dominant leads. (2) The frontal buldging and homolateral occipital flattening correlated most reliably with EEG asymmetries in parieto-occipital leads. Hence, the inaccuracies in the EEG recording seem to matter more for the caudal electrode locations.

Significance to Mental Health Research: The greatest promise of the present approach over the standard (blind) use of the 10-20 system lies in its potential for exploring individual variability of EEG especially in the area of brain laterality where slight asymmetries of EEG amplitude, frequency, and coherence are conceived as related to a varying degree of imbalance of brain reactivity. The interhemispheric distortion is expected to be seen notably in the caudal locations in any diagnostic and/or research effort that requires brain imaging when electrodes are positioned according to the 10-20 system or any other proportionate system. Also, the present study cautions that even an inconspicous measure of skull asymmetry requires that electrode positions are determined on the basis of CT-MRI findings.

Proposed Course of Project: Comparison of electroencephalographic and metabolic brain images would supplement the craniometric approach with an exploration of the effects of fissurization variance on bilateral EEG values.

Publications:

Daniel, D., Myslobodsky, M., Coppola, R., and Weinberger, D.R.: Prefrontal Cortical Atrophy in Schizophrenia is Associated with Skull Asymmetry. Neurosci Abstr. (in press).

Coppola, R., Karson, C., Daniel, D., and Myslobodsky, M.: EEG Asymmetries in Relation to Skull Asymmetries. J. Clin. Neurphysiol., 1987 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02354-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Amphetamine and Frontal Lobe Functioning in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

Principal Investigator: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, IRP, NIMH; Llewellyn B. Bigelow, M.D., Associate Clinical Director, WAW Building, St. Elizabeths Hospital, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research on the effects of amphetamine in schizophrenia is conflicting. It is known that amphetamine may cause paranoid psychosis. On the other hand, transitory improvement in some patients given acute doses of amphetamine has been reported. It is reasonable to speculate that negative symptoms of schizophrenia, such as anergia, lack of motivation, flattened affect, and poor planning may be amenable to such treatment. In addition, patients with schizophrenia generally do poorly on neuropsychological tests thought to assess frontal lobe functioning. We therefore propose a pharmacological strategy involving the administration of amphetamine in the hope that this drug may reverse some of the abnormalities found in chronic schizophrenia, whether they be symptomatic or cognitive.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: It is hypothesized that administration of amphetamine will increase physiological activation in the prefrontal neural system that mediates cognitive activities involving planning, working memory, and responsivity to external stimuli. In addition, subcortical structures that may be affected by amphetamine might manifest activity in a procedural problem solving task and rotational behavior.

Methods Employed: A double blind cross-over design is proposed. Subjects in group one will receive placebo first and five days later an acute oral dose of .25mg/kg dextroamphetamine elixir. Subjects in group two will receive .25mg/kg dextroamphetamine orally and five days later placebo. Following administration, rotational behavior of patients will be assessed by rotometer. One hour after administration of medication or placebo patients will be tested on the Wisconsin Card Sort test, the Tower of Hanoi, Selective Reminding and CPT.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: The study may provide findings that bear on the notion that the prefrontal system in chronic schizophrenia is underactive. In addition, amelioration of such underactivation might have implications for treatment (though not necessarily with amphetamine).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02355-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Autism: A Study of Cerebrophysiology, Neuroanatomy and Neuropsychology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH

COOPERATING UNITS *(if any)*

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Clinical Brain Disorders Branch

SECTION

Section on Clinical Brain

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Few studies have employed multimodal measurements in the same autistic individual, thus considerably reducing their power. The current proposal will attempt to use cerebral blood flow, magnetic resonance imaging, and neuropsychology to provide convergent evidence as to the source of the deficit in autism. In this study, autistic individuals would be presented with cognitive tasks and affect laden tasks in order to challenge various brain regions. Activation will then be correlated with brain structures that were imaged on MR scans as well as results of neuropsychological tests tapping attention, memory, procedural abilities, language, and visual spatial functions. By utilizing autistic individuals with savant skills, we hope not only to gain greater understanding of the neurobiology of exceptional performance, but increased "signal-to-noise" ratio in the autistic population. Autistic individuals without savant skills and mentally retarded individuals, as well as normals, will serve as controls.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description: ZO1 MH 02355-01 CBDB

Objectives: By using cerebral blood flow, magnetic resonance imaging and neuropsychology, we hope to provide converging evidence as to the region or regions of disorder in autism. Furthermore, we hope to gain greater understanding of qualitative and quantitative characteristics associated with savant skills, a domain that has implications for cognitive science, neuropsychiatry, and the study of exceptional abilities.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: We hope the study will have implications for treatment. By carefully delineating areas of normality, hyperactivity or underactivity in the brain, we hope to be able to suggest pharmacological treatments or perhaps even psychoeducational treatments that will ameliorate the disorder. Of course, we hope also, that it permits us to better characterize the nature of autism from a pathophysiological viewpoint.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02356-01 CBDB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Procedural and Problem Solving Abilities in Schizophrenic Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Principal Investigator: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Jean St.-Cyr, Ph.D., Visiting Scientist, Neuropsychology Lab., IRP, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH

COOPERATING UNITS *(if any)*
Neuropsychology Laboratory, NIMH

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section on Clinical Studies

INSTITUTE AND LOCATION
NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Certain tasks that come under the rubric of procedural may be learned by subjects with various organic disorders who otherwise have marked difficulty recalling new information over time. One such tasks is called Tower of Hanoi. It involves moving discs from one peg to another. As well as a procedural component, it also involves problem solving and has been used as a model for conscious problem solving in artificial intelligence. It is thought that the neural system which mediates procedural performance (the basal ganglia) is distinct from the system that is involved in higher level problem solving (prefrontal) or everyday long term memory for items (diencephalic and medial temporal). Two versions of the Tower of Hanoi were administered to schizophrenic patients as well as a control task that involved higher level visual spatial processing. In addition, efforts were made to teach both tasks to patients who experienced difficulty.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: We wish to assess performance in the procedural realm and, if possible, compare it to higher level problem solving that may involve visual spatial components and strategy in planning. The systems at the neural level involved in all three may be distinct. Therefore, direct comparisons might allow one to infer regions of dysfunction and integrity in schizophrenia. In addition, teaching the tasks might shed light on whether deficits, if found, are valid and reflect competence rather than performance. Such a strategy had been used with a prior study of the Wisconsin Card Sorting Test.

Methods Employed: Fifteen normal subjects and 18 patients in the William A. White program who suffered from schizophrenia, participated in the study. Patients were receiving drugs or placebo and manifested tardive dyskinesia, parkinsonism, or no gross movement abnormality. Patients and normal control subjects received a three disc version of the Tower, a four disc version of the Tower, and the block design task from the WAIS-R. Teaching was provided by two methods: a backward chaining stimulus-response paradigm and a cognitively oriented paradigm. Also, subjects were administered multiple trials of the Tower over four days to assess learning.

Major Past Findings: The study is in progress.

Significance to Mental Health Research: The study may improve our understanding of the relationship between cortical and subcortical structures responsible for various aspects of problem solving. Such a finding may implicate a specific neural system, but not others. Moreover, in regard to teaching a block design task, results will bear on a previous study which indicated that schizophrenic patients had grave difficulty learning a task that involved abstraction and set shifting.

Publications:

Goldberg T.E., Weinberger D.R.: Probing prefrontal function in schizophrenia with neuropsychological paradigms. Schizophrenia Bulletin, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02357-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Recall and Recognition Memory in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH; Neil H. Pliskin, M.A., William A. White Division, IRP, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, IRP, NIMH; Marvin Podd, Ph.D., Director of Training, Psychology, Bethesda Naval Hospital

COOPERATING UNITS *(if any)*

Bethesda Naval Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is well known that memory deficits exist in schizophrenia. However, it has been difficult to characterize them. Using the Selective Reminding memory test, 31 schizophrenic patients were assessed on their ability to learn a word list over 12 successive trials (processing which involves acquisition, maintenance and retrieval). These results were compared to recognition memory (in which retrieval or recall factors are minimized) for the word list. Retrieval was differentially impaired and was found to correlate significantly with an anergia factor on the Brief Psychiatric Rating Scale. The latter may be an index of dopaminergic "push." In fact, the profile was consistent with frontal lobe patients who are considered to suffer from the "forgetting to remember" syndrome.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: The study attempted to delineate more completely patterns of memory deficit in schizophrenia. It is believed that there are different neural systems involved in different aspects of memory. By comparing recall to recognition memory and the association of both to psychiatric symptomatology, we believed we would be in a position to draw conclusions regarding differential impairment of specific regions of the brain.

Methods Employed: The Selective Reminding Task was administered to 31 patients with schizophrenia. The test involves reading a list of 12 words over 12 trials. After each trial, only those words that were "forgotten" by the subject were repeated by the examiner. The test yields measures of memory function that include total recall, consistent long term retrieval, and long term storage. In addition, all patients were administered Mini Mental Status Examination and Brief Psychiatric Rating Scale.

Major Past Findings: Recognition memory was relatively intact. In addition, patients manifested a robust learning curve. However, a significant difference between recognition memory and recall memory was noted. Results were consistent with those found in frontal lobe patients. Also, recall memory, but not recognition memory, was correlated with anergia on the Brief Psychiatric Rating Scale.

Significance to Mental Health Research: Schizophrenic patients apparently can acquire new material, but they have difficulty retrieving it. Cognitive rehabilitation programs which aid them in recalling material that has entered their long term store may be helpful. In addition, anergia probably impinges on retrieval functioning. Pharmacological treatments which improve factors involving blunted affect, motor retardation, disorientation and grossly, engagement with the environment, may improve memory functioning as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02358-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Atheoretical Multivariate Statistical Techniques

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Neme, title, leboratory, and institute effiliation)*

Principal Investigators: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH; Neil H. Pliskin, M.A., Staff Psychologist, William A. White Division, IRP, NIMH, Karen F. Berman, M.D., Staff Psychiatrist, CBDB, IRP, NIMH; Marvin Podd, Ph.D., Director of Training, Psychology, Bethesda Naval Hospital

COOPERATING UNITS *(if any)*

Bethesda Naval Hospital

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multivariate data reduction techniques have recently become available through statistical computer packages. Through both factor analysis and cluster analysis, variables and cases respectively may be aggregated. These techniques were applied to a sample of schizophrenic patients who had received the Wisconsin Card Sort. In the latter study, data that were based on means were employed. However, the approach might have obscured distinct subtypes. Therefore, we attempted to cluster patients based on performance. However, we found that even small changes in the statistical programs led to major differences in the solutions we derived.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description: Z01 MH 02358-02 CBDB

Objectives: We wished to investigate whether subtypes were present in schizophrenic patients' performance on the Wisconsin Card Sort and their response to instructions to the Wisconsin Card Sort. Also included in these data were Selective Reminding memory scores, Mini Mental Status scores, and Brief Psychiatric Rating scores. We subjected these data to various forms of factor analysis and cluster analysis in an effort to delineate subtypes. We hoped these would have clinical relevance.

Methods Employed: SAS programs were used. Principal component, orthogonal varimax, and oblique promax transformations were used to reduce the number of variables. After these analyses, cluster analysis, using Wards minimum variance procedure, was employed. Also, rather than using factor scores in analyses, raw scores were also used. Twenty-four cases were entered into the study.

Major Past Findings: Small changes in the statistical analyses produced major differences in interpretation. With one analysis (involving factors and then cluster analysis) subtypes emerged. However, other analyses (in which raw scores were used or factors based only on Wisconsin Card Sort performance were used) yielded no subtypes as based upon an objective for determining cluster number. These results indicate that atheoretical statistical techniques in which there are few means to reject or accept results must be employed cautiously.

Significance to Mental Health Research: When there is distance between the researcher and data, caution must be exercised when utilizing sophisticated and elegant quasistatistical multivariance techniques. Without subjecting the data to more than one technique, a very different solution to our problem might have been arrived at. Before accepting any one solution, which may or may not be more valid than the next, consistency across techniques should be sought. This methodological issue has ramifications for research on a wide variety of neuropsychiatric problems.

Publications:

Goldberg T.E., Weinberger D.R., Berman K.F., Pliskin N.H., Podd M.H., Further evidence for dementia of the prefrontal type in schizophrenia. Archives of General Psychiatry (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02359-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Age Disorientation, Mental Status, and Ventricular Brain Ratio

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH

Others: Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, IRP, NIMH; Michael S. Myslobodsky, M.D., Visiting Scientist, CBDB, IRP, NIMH; David G. Daniel, M.D., Medical Staff Fellow, CBDB, IRP, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

A sizable minority of schizophrenic patients are age disoriented, that is, they do not know their own chronologic age. This special type of impairment was not found to be associated with enlarged cerebral ventricles by another group. However, the mean age of their group was old and they did not distinguish patients delusional for their age from patients demented and age disoriented. We assessed a younger sample and separated age delusional from age disoriented demented patients and found a significant difference in ventricular-brain ratio (VBR) between age oriented and age disoriented/demented patients.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: This study investigated the neuroanatomic correlates of global cognitive failure in schizophrenic patients. When, in fact, global cognitive failure included age disorientation, ventricular enlargement may be a frequent concomitant.

Methods Employed: Former and current William A. White patients between the ages of 20 and 60 who had been diagnosed as schizophrenic were eligible for the study. All subjects were asked orientation questions that included probes regarding their age. Also, all patients received the Mini Mental Status Examination, a cognitive screening instrument that involves linguistic, visual spatial, praxic, and memory abilities. Of the 39 patients in the study, 27 had received CAT scans during their hospitalization.

Major Past Findings: Eight of 39 patients did not know their correct age. Of these, six were demented and age disoriented (as based upon Mini Mental Status score) while two were delusional (as based upon Mini Mental Status score and interview). All six patients with age disorientation and dementia had ventricular brain ratios greater than 2 sds above the mean of a control group. In contrast, 14 of 19 patients without age disorientation and dementia had ventricular-brain ratios less than 2 sds above the mean.

Significance to Mental Health Research: This study demonstrated that when a patient is age disoriented and demented, the probability of structural abnormality is high. Thus, when cognitive failure is global rather than differential, gross atypicalities in the brain may be imaged.

Proposed Course of Project: The project has been completed in its first phase. It is hoped, however, that age delusional subjects can be recruited and studied so that more may be learned about fixedness of ideas in schizophrenia. Also, the study may be expanded to replicate the original finding in a larger group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02360-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Topographic Analysis of Brain Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Richard Coppola, D.Sc., Senior Engineer, CBDB, IRP, NIMH

Others: Richard K. Nakamura, Ph.D., Senior Staff Fellow, LNP, NIMH; Robert M. Cohen, M.D., Ph.D., Chief, CBI, LCM, NIMH; David Pickar, M.D., Chief, SCS, NSB, NIMH; Robert M. Post, M.D., Chief, BPB, NIMH; Rex Cowdry, M.D., Clinical Director, NIMH; Richard J. Wyatt, M.D., Chief, NPB, NIMH; John M. Morihisa, M.D., Chairman, Department of Psychology, VAMC, Washington, D.C.; Trey Sunderland, M.D., Medical Officer, LCS, NIMH; Judith M. Rumsey, Ph.D., Senior Staff Fellow, CPB, NIMH

COOPERATING UNITS *(if any)*

LNP, NIMH; LCM, NIMH; NSB, NIMH; BPB, NIMH; VAMC, Washington, D.C.; LCS, NIAAA; CPB; EB, NINCDS; LNS, NIA; DEB, NICHD; Columbia University, NY; VA, Duke University, N.C.; Carter Memorial, IN; AFB, Berlin, W. Germany

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrical brain activity, as an index of central nervous system function, is studied across a range of patient groups with neurological and psychiatric disorders as well as normal volunteers. Using electrophysiological data quantified from event-related potentials and spectrum analysis of EEG recordings, computer-derived brain images are able to provide information about neurophysiological function relating to both cognition and clinical state. Topographic maps efficiently characterize spatial and temporal patterns of brain activity allowing the ability to study the dynamic interaction among brain regions and their relation to function.

The project has two main purposes. The first is to refine the topographic and quantitative analysis methods and establish normative data for various conditions and activation procedures. For example, normal subjects differ with respect to their major focus of resting EEG alpha rhythm; one group shows a dominant parietal locus and one an occipital locus, depending on the alpha frequency.

The second purpose is to apply these methods to the characterization of clinical groups and pharmacological response. Work in progress includes characterization of subgroups of Alzheimer's patients, localization of abnormality in epilepsy patients, localization of drug activation and study of psychiatric patients on various neuroleptic drugs..

PHS 6040 (Rev. 1/84) GPO 914-918

Continuation of Investigators:

Others: William H. Theordore, M.D., Research Neurologist, EB/NINCDS; Mark Hallett, M.D., Clinical Director, NINCDS; Markku Linnoila, M.D., Chief, LCS, NIAAA; Stanley I. Rapoport, M.D., Chief, LNS, NIA; Susan R. Rose, M.D., Medical Officer, DEB, NICHD; Harold Sachheim, Ph.D., Columbia University, New York; Richard D. Weiner, M.D., Ph.D., VA, Duke University, N.C., Victor Milstein, Ph.D., Carter Memorial, Indianapolis, Indiana; Werner Herrman, M.D., AFB, Berlin, W. Germany

Objectives: The overall goal of this project is to develop and apply methods for utilizing the electrical activity of the brain as a measure of central nervous system function with the expressed purpose to study human information processing, including attention, sensory processing, and cognition and to study functional states as seen during chronic or transient conditions. Methods have been developed to display topographic maps that efficiently characterize brain activity in terms of both spatial and temporal patterns. The ability to study these patterns in a dynamic fashion will yield a better understanding of the interaction among brain areas and their relation to function.

The project has two main thrusts. The first is to refine the topographic and quantitative analysis methods and establish normative data for the patterns of brain activity seen in a variety of conditions and under various activation procedures. Differentiation of EEG patterns associated with cognitive and attention-related parameters are of importance to understand the underlying neurophysiological basis of both normal and abnormal brain function.

The second thrust is to utilize these methods and normative base to discover and describe characteristic brain activity patterns in a variety of patient groups. The main hypothesis is that certain patient groups will exhibit regional localization of EEG abnormalities. It is expected that quantitative topographic analysis will provide better sensitivity for this localization than the usual clinical EEG recordings. An additional hypothesis is the expectation of changes in quantitative EEG parameters with treatment in patient groups.

Methods Employed: Multilead scalp recordings are made during baseline resting conditions and during various activation procedures. Quantitative reduction of this data is performed by computer spectrum analysis to rpovide a profile of the energy in the different frequency bands of the EEG. Combining this data with an equal area projection of the scalp surface gives a computer-generated display of a map of brain activity. The map is used to define a baseline condition and changes in the map are used to assess regional patterns during activation procedures. Maps of the raw EEG itself are used to follow the temporal and spatial development of specific EEG events such as a spike and wave complex. Event-related potentials (ERPs) are collected to visual pattern stimulation. Maps are made in a similar fashion for this data and used to determine the intactness of sensory pathways.

Methods employed in specific clinical studies fall into two categories. The first type is where a patient is seen only once. In this case, comparison with other clinial groups or normative data is used to derive characteristic topographic profiles. Correlation or subtyping, using neuropsychological assessment from other studies, may also be carried out. In the second case, patients are seen more than once and assessment is made in regard to change in clinical state, medicaiton, or other treatment.

Collaborative Centers: Because of considerable interest in the research community and as a means to refine these methods and expand the available data base, several collaborating laboratories are now using the system we have developed. This includes laboratories in NINCDS and NIAAA, as well as several outside the NIH.

Major Past Findings: Methodology: A major cause for concern in topographic mapping has been the choice of reerence electrode. We have developed

re-referencing methods and the use of a Laplacian transform to produce a reference-free, source-density may to deal with this issue. Utilizing EOG electrodes to monitor eye movements has removed this troublesome artifact.

A set of activation procedures for simple vigilance and a verbal and spatial memory task have been developed. Asymmetry of EEG response has been shown in several groups using these tasks.

Normative Studies: Currently at issue is whether normative topographic maps can be developed. We have shown that as regards the alpha band, there are four clusters of normals rather than a simple normal distribution. Subjects can be classified into two main subgroups. Those subjects with peak alpha frequency below 10.2 Hz have a parietal pattern and those above 10.2 Hz an occoiptal pattern. This suggests two different generators. It is unclear, as yet, as to whether or not these differences reflect salient neurophysiological or neuropsychological characteristics of the subjects

Pharmacological Characteristics: A study of a double blind crossover of placebo, amitriptyline, chlorepromazine, and diazepam has shown specific regional effects in addition ot the usual spectrum differences due to these drugs. These data are being used as a model to investigate the complex multivariate statistics needed to analyze these studies. An investigation of nootropic agents has shown that these do not follow a specific EEG profile of changes, but rather depend on characteristics of the baseline EEG.

Epilepsy: Recordings from more than 40 seizure patients have been completed. Some characteristic patterns have emerged from the variety of disorders represented. The dynamic pattern of the spike and wave complex was seen to be almost identical in petit mal patietns as well as other spike-wave. In all cases, the spike has a mid-line frontal maximum that does not shift position during the time course of the spike itself.

A comparison of quantitative EEG to other neuro-imaging methods has shown good agreement, even in several cases when routine surface EEG was equivocal.

Other Clinical Studies: In a comparison of dyslexic adults and normals, the dyslexic group showed less asymmetry during cognitive activation tasks while they had greater asymmetry during rest. These data will be compared with other data collected on this group.

Normal volunteers receiving steroids have shown theta EEG increases. Comparisons to other clinical measures on this group are being studied.

Significance to Biomedical Research and to the Program of the Institute: Electrical activity recorded at the scalp is currently the only non-invasive technique available as a window on the physiological functioning of the human brain. While EEG is a very indirect measure of neural activity, its advantage is the ability to reflect changes on a millisecond-by-millisecond basis. This allows EEG to be related to behavior in an ongoing manner. In contrast, PET images have higher resolution and more directly measure neural activity but reflect the summation of activity over a period of correlation of activity with behavior. EEG imaging gives complementary data to the other modalities of cerebral metabolism (PET) and blood flow (rCBF).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02388-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 charecters or less. Title must lit on one line between the borders.)*

Regional Cerebral Blood Flow in Neuropsychiatric Patients and in Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigetor.) (Name, title, laboretory, and institute affiliation)*

Principal Investigators: Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, IRP, NIMH and Karen Faith Berman, M.D., Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH

Others: Douglas Jones, Ph.D., Physicist, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS *(if eny)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.25	2.25	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do hot exceed the space provided.)*

Using the Xenon133 inhalation technique, the regional cerebral blood flow (rCBF) lab within the Clinical Brain Disorders Branch carries out investigations of rCBF (as an indicator of regional cortical metabolism) in a variety of neuropsychiatric patients and in normal subjects. Patient populations, including those with chronic schizophrenia, affective disorder, obsessive-compulsive disorder, Huntington's disease, Parkinson's disease, Alzheimer's disease, and dyslexia are studied before and during various exploratory and therapeutic interventions. Normal control subjects matched for each patient study are investigated concurrently. Cortical metabolic concomitants of states of normal cognition and consciousness are also being explored. The Xenon133 method allows for multiple determinations of rCBF in a single individual who can thus serve as his or her own control while being studied serially under various cognitive and/or medication conditions. This allows paradigms to be designed to specifically test hypotheses about regional cortical function in disease states and normal higher cognitive function, and to specifically monitor experimental and therapeutic interventions in neuropsychiatric disorders. Careful and creative application of this versatile tool has produced important results. Experiments tailored to explore dorsolateral prefrontal cortex (DLPFC), an area of special interest in schizophrenia, have shown this area to be de-activated in patients with schizophrenia under conditions of cognitively specific, regionally selective demand upon this area - conditions under which normals increase metabolism in DLPFC. In contrast Huntington's disease patients, who are as cognitively impaired as schizophrenics, do not show DLPFC rCBF abnormality, but rather rCBF patterns similar to normal subjects. This is important evidence for the existence of subcortical dementia, which, until now, has been questioned by some.

Project Description:

Objectives: Until recently, investigations of the human brain have been restricted to animal model facsimiles, subjective clinical observations of human subjects, and measurements of peripheral markers of CNS activity in the blood, urine, or CSF. However, new techniques for directly determining brain function in living human patients are now yielding important data about regional neurophysiology in normal function and in disease states. One such technique, Xenon133 inhalation rCBF, has proved particularly well suited to the study of neuropsychiatric disease. With this method, which has been shown to be tightly linked to local metabolic activity, rCBF can be measured in various patient populations in a relatively expedient, convenient, reliable, and inexpensive manner, and regional physiological correlates of normal brain function and neuropsychiatric disease can be determined. This is an essential prerequisite to the understanding and treatment of these disorders and has important potential implications for monitoring therapeutic interventions.

Using this technique, the NIMH rCBF lab is endeavoring to delineate the regional neurophysiological concomitants of 1) normal brain function and aging, 2) neuropsychiatric illnesses including schizophrenia, affective disorder, multiple personality disorder, obsessive-compulsive disorder, Parkinson's disease, Huntington's disease, Alzheimer's disease, and dyslexia, and 3) the sequelae of such brain insults as encephalitis and leucotomy. In addition to investigations of cortical function in these conditions, we are also undertaking the direct study of effects of pharmacotherapeutic interventions on cortical physiology and their relations to clinical response. These latter studies include evaluations of effects of standard medications, such as neuroleptics and anticholinergics in schizophrenia and levodopa/carbidopa in Parkinson's disease, as well as novel experimental treatments such as calcium channel blockers, arginine-vasopressin, and dopamine agonists in schizophrenia, and the muscarinic agonist, RS-86, in Alzheimer's disease.

To maximize the contribution of the regional neurophysiological data obtained, the rCBF team works with other investigators to build a multimodal data base consisting not only of rCBF data, but also correlative neurostructural data (via CT scanning and NMR), clinical and demographic information, other complementary measures of neurophysiology (PET and BEAM), and cognitive batteries.

Methods Employed: Regional cortical metabolism is determined using radioactive tracer kinetics principles. Following a one-minute inhalation of small concentrations (5-7 mCi/liter) of the physiologically and chemically inert and freely-diffusable radioisotope gas, Xenon133, rates of arrival and elimination of radioactivity in 32 cortical areas are monitored via an array of extracranial sodium iodide scintillation detectors for an additional 14 minutes. Subjects are typically studied under three different cognitive conditions during a single morning or afternoon session. The first of these is a "resting state" study primarily for purposes of acclimatization. Then rCBF is measured during two cognitive activation conditions that are presented in counterbalanced sequence. Usually these are paired tasks, one of which is tailored to activate the cortical region of interest to the disorder, therapeutic intervention, or normal function being investigated. The other task serves as a control for those aspects of the procedure that are non-specific and extraneous to the study. Various parameters of autonomic arousal including galvanic skin response, pulse, respiratory rate, and carbon dioxide level are monitored during each procedure.

Paradigms are designed to address specific research questions. The following are examples of past and on-going paradigms (each of which is performed while rCBF is measured, as is its specially designed control procedure): (1) to study dorsolateral prefrontal cortical function, an automated version of the Wisconsin Card Sort (WCS) designed in this laboratory (on-going in schizophrenia, affective disorder, Huntington's disease, Parkinson's disease,and post-leukotomy patients),(2) to assess cortical concomitants of attention and mental effort, two versions of a visual continuous performance task (CPT) that differ in difficulty and the amount of sustained effort and attention required (on-going in normal subjects and schizophrenia during various medication protocols), (3) to assess non-regionally specific complex reasoning and medication-effects, split-pair automated versions of Raven's Progressive Matrices (RM) that can be carried out on consecutive days without learning effects (on-going in schizophrenia, Alzheimer's disease and Parkinson's disease during medicated and unmedicated states), (4) to assess cortical laterality, automated semantic classification and line orientation tasks (on-going in dyslexia and normal subjects), (5) to study the cortical effects of emotional state, simulated anxiety/depression paradigms (carried out on trained psychodramatists), (6) to assess temporal lobe function in schizophrenia, an auditory discrimination task.

Single Photon Emission Computed Tomography (SPECT), a new technology that allows rCBF of deep brain structures to be measured, is currently being set up in the Clinical Brain Disorders Branch.

Major Findings: Schizophrenia: A number of observations implicate dorsolateral prefrontal cortex (DLPFC) in schizophrenia, including clinical symptoms similar to those of frontal lobe disease, decreased relative DLPFC regional cerebral blood flow (rCBF), and animal studies suggesting a role for DLPFC in cognitive processes that are commonly impaired in chronic patients.

rCBF was measured with Xenon 133 inhalation during various cognitive tasks. First, to assess DLPFC function, 20 patients medication-free (DF) for at least four weeks, 24 on medication, and 25 normal subjects completed a three-test series. rCBF was determined initially during the resting state, then while subjects performed, in counterbalanced sequence, an automated version of the Wisconsin Card Sort (WCS) to selectively test DLPFC, and a simple numbers matching task (NM) to control for non-DLPFC related aspects of the procedure. Next, to assess the roles of attention and task specificity in 17 DF patietns and 18 controls during two versions of a visual continuous performance task (CPT), an attentional task not specific for DLPFC. Finally, to further determine DLPFC function during complex but non-DLPFC-linked reasoning, rCBF studies were done while subjects solved Raven's Progressive Matrices (RM). Brain structure was assessed with CT, in 18 DF and 22 medicated patients who completed the WCS/NM paradigm.

DLPFC rCBF was selectively decreased in DF and medicated patients specifically during WCS. No DLPFC difference between patients and controls was noted during NM, CPTs or RM. Degree of DLPFC activation was correlated with patients' performance on the WCS but not with autonomic arousal. DLPFC rCBF correlated with several parameters of structural pathology in CT. These data suggest a pathophysiological mechanism for the cognitive impairment in schizophrenia.

The WCS-related DLPFC deficit in schizophrenia has also been found in a new cohort of 16 DF patients.

Huntington's disease: The dementia of Huntington's disease (HD), though clinically familiar, has been difficult to characterize on a neuropathological basis. Despite the long-held assumption of the importance of cortical degeneration, evidence of a link between dementia and cortical atrophy is weak. PET studies of glucose metabolism in patients with HD suggest that, in contrast to striatum, cortex is normal in the resting state and during simple motor movements. The present study is an investigation of rCBF in patients with HD during performance of a cognitive task on which they characteristically do poorly.

Ten patients (mean age + S.D.; 41 + 12) and 15 normal volunteers underwent three Xenon133 inhalation rCBF procedures: first at rest and then, in counterbalanced sequence, while performing the WCS, which selectively tests DLPFC, and while performing a NM task, which served as a control. At rest and during NM, grey matter CBF to prefrontal (DLPFC) and precentral regions both in absolute levels and as a ratio of non-frontal flow did not differ significantly between groups. Despite many more perseverative and conceptual errors on the WCS, the HD patients' prefrontal and precentral CBF was not different than normals during the test. Likewise, regional CBF % changes (WCS-NM)/NM did not differ significantly between groups. In contrast, stage of illness (Shoulson and Fahn), while correlating with WCS performance, did not correlate with rCBF. These results, by suggesting normal cortical metabolism during abnormal cognitive function, provide additional evidence for the role of subcortical pathology in the dementia of HD. Because of caudate pathology, prefrontal cortex is presumably de-efferented.

Parkinson's disease: Dopaminergic projections from midbrain to prefrontal cortex have been implicated in the monkey in certain cognitive functions mediated by prefrontal cortex, e.g., delayed response tasks. While the role of these projections in human cognitive function is less clear diminished mesoprefrontocortical dopaminergic activity is found in Parkinson's disease, as are cognitive deficits suggestive of prefrontal cortical dysfunction, e.g., difficulty with problem solving. The purpose of the present study was to investigate prefrontal physiology (i.e., rCBF) and cognitive function simultaneously in patients with Parkinson's disease, both before and after dopamimetic treatment.

Ten medication-free patients (mean age + S.D.; 63.9 + 2.8) with idiopathic Parkinson's disease (stage I=3, II=4, III=2, IV=1) who had never received dopamimetic agents and 11 similarly aged normal volunteers underwent in counterbalanced sequence two Xenon-133 inhalation rCBF procedures: once during performance of WCS, a test of prefrontal cortex (PFC) cognitive function that in normals selectively increases PFC rCBF, and once during NM which served as a baseline. These procedures were repeated in the patients after three months of optimum doses of L-dopa/carbidopa.

Compared with normals, patients had a slight (~15%) but nonsignificant reduction in mean brain blood flow during both conditions; L-dopa treatment did not significantly increase rCBF. It did, however, affect the pattern of rCBF during the cognitive tests. Prior to treatment, patients tended to show generalized cortical activation during the WCS; this became more selectively prefrontal (i.e., more like normal) after treatment. Furthermore, the degree of PFC activation during the WCS (i.e., PFC rCBF during WCS divided by PFC rCBF during NM) correlated with performance on the WCS ($r=-.73$, $p<.01$), and also with stage of illness ($r=-.80$, $p<.01$), degree of rigidity ($r=-.66$, $p=.05$) and bradykinesia ($r=-.65$, $p<.06$), but not with tremor, age, or duration of illness.

These data strongly suggest that dysfunction of prefrontal cortex underlies some of the cognitive symptomatology of Parkinson's disease and the reduced dopamine afferentation may be the pathophysiological mechanism. This supports the notion that mesoprefrontocortical dopaminergic projections are involved in PFC physiological activation and cognitive behavior.

Alzheimer's disease: New techniques for measuring regional brain physiology in vivo may be useful adjuncts for diagnosing neurodegenerative disorders such as Alzheimer's disease (AD) and for understanding the pathophysiology underlying them. However, previous studies have not been consistently successful in distinguishing patients from elderly controls.

In the current study 20 patients with AD (mean age 63 years, range 55-68) and 15 normal control subjects matched for age, sex, and education had rCBF measured by the Xenon-133 inhalation method. rCBF was determined during three different testing conditions: first during an eyes-closed resting state and then during two cognitive activation tasks, one an automated version of Raven's Matrices (RM), a task linked to posterior association cortex, and the other a similarly automated Number Matching (NM) baseline task. The order of the two activation tasks was counterbalanced across subjects.

During all three testing conditions, gray matter rCBF for every cortical area averaged 20% lower in patients with AD than in normal subjects ($p<.03$). However, during RM, both groups increased posterior rCBF over that during the baseline NM task. During the RM and the NM, but not during resting, clinical status (as assessed by the Mini-Mental State exam) correlated with rCBF values for several cortical areas.

Discriminant function analysis was performed on the rCBF values derived during each task to create a mathematical model that best differentiated the patients from the normal group. Each model was then retrospectively applied to classify each subject into one of the two subject categories (patient vs. normal), and the relative success rates for each model were assessed.

In the retrospective discriminant function analysis, rCBF for each of the three testing conditions correctly classified the majority of the patients and normals into the appropriate diagnostic category. Compared to the resting state, cognitive activations improved the ability of the rCBF models to distinguish the two groups (during resting 89% of the normals and 79% of the patients were correctly classified; during RM 89% of the normals and 89% of the patients; and during NM 93% of the normals and 89% of the patients). Discriminant function models derived from rCBF during all three conditions retrospectively classified subjects with 100% accuracy.

These data are consistent with previous reports of decreased cortical activity in patients with AD. These findings further suggest that measuring cortical activity during several different conditions, including cognitive activation, improves the ability of physiological measures such as rCBF to differentiate patients with AD from controls.

Significance to Mental Health Research: The regional pathophysiology underlying neuropsychiatric disorders, and even that accompanying normal higher cognitive function or emotion, is poorly understood. The rCBF lab within the Clinical Brain Disorders Branch has a unique opportunity to directly study CNS phenomena in a variety of clinical populations, to elucidate normal neurophysiology and to directly monitor the CNS effects of therapeutic interventions. Furthermore, by

elaborating a multimodal correlative data base of cognitive, structural, and clinical measures we are better able to interpret our rCBF data from a broader perspective. The opportunity to search for commonalities and differences in a wide variety of patient populations and clinical states maximizes the potential of this work to delineate the regional underpinnings of CNS pathophysiology as well as normal function. Thus, our ultimate goal is to better understand the until-now elusive workings of the living human brain in health and in disease states, and to successfully intervene in the latter.

Proposed Course of Project: The first rCBF procedure was carried out in March, 1983, and since that time over 2000 individual procedures have been successfully completed in the rCBF lab. Data management techniques for the resultant extensive informational base and statistical methods to address the complex interrelationships of regional brain function have been and continue to be developed. Important research questions have been formulated, and in some cases the answers have led to new questions. In addition to the continuation of the projects described above, new protocols to evaluate the effect of dopamine agonists in conjunction with neuroleptics in schizophrenia are anticipated.

Implementation of the new SPECT technology will allow the subcortical correlates of these findings to be studied.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02389-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Brain Electrical Activity Mapping in Neuropsychiatric Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Craig N. Karson, M.D., Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH

Others: Terry Goldberg, Ph.D., Special Expert, CBDB, IRP, NIMH; Karen F. Berman, M.D., Staff Psychiatrist, CBDB, IRP, NIMH; Ralph Fawcett, M.D., Medical Staff Fellow, NPB, IRP, NIMH, Richard Coppola, D.Sc., Senior Engineer Officer, CBDB, IRP, NIMH; Daniel R. Weinberger, M.D., Chief, CBDB, IRP, NIMH

COOPERATING UNITS *(if any)*

Neuropsychiatry Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have confirmed that patients with schizophrenia have an increase of slow wave activity (delta) throughout the brain and a left sided increase in fast (beta) activity. The peak frequency is slowed in schizophrenia and this slowing is related to increased cerebral ventricular size.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives: We continue to clarify the EEG abnormalities in schizophrenia in an effort to examine the possible brain mechanisms related to schizophrenia.

Methods Employed: The subjects are patients with chronic schizophrenia withdrawn from medications for four weeks as well as other patients with brain disorders of interest. Normal controls are recruited from clinical staff and newspaper advertisements.

Twenty-eight gold electrodes referenced to linked ears record scalp EEG activity. Two electrodes, one on each outer canthus, referenced to each other, provide the horizontal electrooculogram to study horizontal eye movements. All signals are sent through Grass amplifiers then to a PDP11/23 for A to D conversion, fast fourier transform, mapping and filing for statistical analysis (SAS software, multivariate analysis of variance, MANOVA).

The EEG is recorded during the resting state.

Major Past Findings: Our predecessor in this laboratory found the patients with schizophrenia had increased bifrontal delta activity which correlated to frontal atrophy on CT scan.

New Findings: Patients with schizophrenia have more slow wave activity and left sided fast wave activity. The dominant frequency is slowed which correlated to increased cerebral ventricular size.

Significance to Mental Health: The EEG is the first non-invasive technique for studying the dynamic events in the brain. Its time resolution in μsecs and its total safety makes it an ideal research tool in all mental disorders, especially with the more comprehensible conversion of the data into maps. Now that we are more clear what the actual EEG abnormalities are, it may be possible to relate these abnormalities to the brain mechanisms associated with schizophrenia. The new findings of alpha slowing and its relation to cerebral ventricular size will be presented at the International Congress in March of 87.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02390-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

An Exploration of Parietal Functions in Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Michael Myslobodsky, M.D., Clinical Brain Disorders Branch, NIMH

Others: Terry Goldberg, Ph.D., Clinical Brain Disorders Branch, NIMH, Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.1	3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have found that schizophrenic patients show imitative dyspraxia of manual sequences and have difficulty in imitating facial sequences. Also, they are less competent than controls matched for sex, age, and education in lipreading of lexcal items overlearned sentences, but not words and syllables. These findings implicate the parietal lobe. Since the neuropsychology of schizophrenia is commonly conceived as akin to the "frontal lobe syndrome" these findings call attention to the possibility that the involvement of the parietal area in schizophrenia may be greater than formerly thought.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: Schizophrenic patients and their offsprings are frequently motorically deficient, clumsy, and exhibit awkward finger movements. Numerous studies have supported Liepmann's (1908) original claim that bilateral gestural behaviors share the neuronal command system with the one underlying speech. We therefore explored a possibility that speech tested in the visual modality (lipreading) would be deficient concurrently with signs of dyspraxia exhibited by schizophrenic patients.

Methods Employed: A paradigm of Kimura (1982) validated in normal individuals and brain damaged patientrs, was employed to juxtapose manual postures and oral grimaces with imitation of syllabic and multisyllabic speech. It was modified to tests receptive speech competence in the same (visual) modality by examining lipreading. Fifteen patients who answered the DSM-III criteria for schizophrenia were assessed for (1) Handedness; (2) Apraxia; (3) Persistence; (4) Single and sequential oro-facial mimicry; (5) Single and multiple hand postures; (6) The susceptibility for blend illusion; (7) consonant recognition test (CRT); (8) Overlearned (cliche) phrase recognition test (PRT).

Major Past Findings: There were indications mentioned by our group and others that schizophrenic patients have unusual difficulty in imitating the non-communicative manual sequences. There were no attempts to conceive this phenomenon as a part of parietal lobe dysfunction. Also, no studies examined lipreading competence in schizophrenia.

New Findings: (1) All patients did well on isolated manual gestures and facial grimaces. They were inferior on facial sequences, but were more noticably disabled in reproducing manual sequences, even sequences with recognizable symbolic hand postures. Those who scored high on facial sequences and/or pantomime were still capable of only crude approximation of three-step manual sequences.

(2) On overlearned phrase recognition test, patients were significantly inferior to controls. In the latter test, 70% of patients evinced a number of responses that were not contained in the target material ("errors by enrichment").

Significance to Mental Health Research: Imitative dyspraxia of manual sequences seems to be far more robust, easy to quantify, and stable phenomenon than any motor "soft sign" seen in schizophrenia. Together with lipreading deficit, praxis functions difficulties may be conceived as supplementing an obligatory bond between dyspraxia and aphasia. The meaningless manual sequences along with speech analysis in the visual modality seem to be subserved by the dominant parietal lobe suggesting a deficit of that area. Contemporary research efforts in designing a better drug for schizophrenia continue to revolve almost exclusively around dopamine antagonists. If parietal lobe deficit in schizophrenia is confirmed, the necessity to reconsider the contribution of other neurotransmitter systems (e.g., GABA/benzodiazepine system) may be more compelling.

Proposed Course of Project: Two parallel and independent studies are expected to advance this project. They are (1) replication of manual dyspraxia and lipreading difficulties in a larger sample with an emphasis on sex-differences of the above effects. (2) An investigation of the rCBF during lipreading efforts in control and schizophrenic patients.

Johnson, F., Hicks, L., Goldberg, T., Myslobodsky, M.S.: Sex differences in lipreading. Bull. Psychon. Soc. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02391-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Phenomena in Schizophrenia and the Development of Novel Treatments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principal Investigator: Craig N. Karson, M.D., Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH

Others: David G. Daniel, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch, IRP, NIMH, Llewellyn B. Bigelow, M.D., Clinical Director, WAW Division, Saint Elizabeths Hospital, IRP, NIMH; Darrell G. Kirch, M.D., Associate Clinical Director, NPB, IRP, NIMH; Joel E. Kleinman, M.D., Ph.D., Deputy Chief, Clinical Brain Disorders Branch, IRP, NIMH

COOPERATING UNITS *(if any)*

Neuropsychiatry Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.6	.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Despite wide variations in the clinical manifestations of schizophrenia, it remains difficult for clinicians to design novel treatments or predict vulnerability to negative outcomes (including medication side effects) based on signs and symptoms of the disorder. By focusing on certain clinical characteristics of schizophrenia, we have designed a series of experimental medication treatments. This involves the notion that the disorder is associated with impairment cognitive performance. Hence, we have begun trials of medication that may improve cognition. Our first such trial utilized DDAVP with at least modest success. We are in the process of completing a trial of hydergine, another medication associated with improved cognition.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description:

Objectives: To describe and quantitate the relationship of clinical manifestations of schizophrenia to the outcome and treatment of schizophrenia and to use related concepts to design new types of treatment.

Methods Employed: 1) Medication treatments: We have been interested in trying medications that might enhance cognitive function in schizophrenia as this disorder been associated with impaired cognition. The first such medication trial involved DDAVP, and we are about to undergo a similar trial with Hydergine. These are both double blind placebo controlled studies of subjects with chronic schizophrenia either in conjunction with ongoing neuroleptic treatment or while these patients are mediation free. The total duration of each study is approximately three months. Behavioral change is quantitated by both the Brief Psychiatric Rating Scale (BPRS), as measured daily by trained nursing staff and a negative symptom rating scale developed in this laboratory.

Major Past Findings: 1) DDAVP produced modest improvement in patients with schizophrenia predominantly in the so-called "negative" symptoms.

New Findings: Clinical studies with Hydergine are nearly completed.

Significance to Mental Health Research: Many patients with chronic schizophrenia respond poorly to neuroleptic treatment or develop serious side effects such as TD or neuroleptic malignant syndrome. Our promising results with DDAVP suggest another avenue to approach this disorder namely as a cognitive impairment. While DDAVP was associated with different, albeit serious side effects, other, safer cognitive enhancing agents may prove effective as well, perhaps providing an alternative to neuroleptic therapy. The import of a successful trial of Hydergine is self evident.

Proposed Course of Project: The results from the Hydergine trial are to be presented at Biological Psychiatry, May '87.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02392-01 CBDB

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation Of Patients With Prefrontal Leukotomies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Karen Faith Berman, M.D., Staff Psychiatrist, Clinical Brain Disorders Branch, IRP, NIMH; Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch IRP, NIMH; Daniel R. Weinberger, M.D., Chief, Clinical brain Bisorders Branch

Denise Juliano, MSW,Social Worker, Clinical Services Branch, WAW Division
Richard Suddath, M.D., Medical Staff Fellow, NPB,DIRP,NIMH; Terry E. Goldberg, Ph.D., Special Expert, CBDB,IRP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section On Neuropathology Studies

INSTITUTE AND LOCATION
NIMH,WAW Bldg., St. Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
10	6	2

CHECK APPROPRIATE BOX(ES)
- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Prefrontal lobotomies for psychiatric illness were last performed in the 1950's. Approximately 40 patients who had dorsolateral prefrontal lobotomies remain on the patient rolls at St. Elizabeths Hospital. The long-term effects of prefrontal lobotomy and the overall outcome of the patients is not clearly known. Techniques are now available to study anatomic, clinical and physiological aspects of lobotomy patients. These include computerized tomography scanning (CT), magnetic resonance imaging (MRI), and cerebral blood flow (cbf) examination. Neurological, psychiatric, and psychological evaluation of these patients in correlation with the above examinations compared to age and diagnosis matched-non-lobotomized patients, will give information on both the effects of prefrontal lobotomy and on the function of prefrontal cortex.

PHS 6040 (Rev 1/84)

GPO 914-918

Project Description:

Objectives: Detailed physiological, clinical, and anatomic study of patients 30-40 years after prefrontal leukotomy have not been done. This study will add to the fund of knowledge on the long-term effects of lobotomy, and, more generally, contribute to understanding frontal lobe function.

Methods Employed: Patients who underwent prefrontal lobotomies have been identified either from St. Elizabeths records or from recognition of typical lesions on CT (computerized tomography) scans obtained for other reasons. Controls are being selected from the St. Elizabeths roster and matched for age, diagnosis, and duration of hospitalization. The patients receive a neurological examination and neuropsychological battery. CT and MRI scans are used to define the lesions. Cerebral blood flow evaluation using Xenon 133 inhalation during the performance of cognitive tasks provides a measure of physiological function.

Major Findings: The study is in progress. Significance to Mental Health Research
The study will yield information on outcome in patients who underwent prefrontal lobotomy. It will also yield information on frontal lobe function.

Proposed Course Of Project: The project will run until all known lobotomy patients who are able to participate have been examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02393-01 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Demeclocycine In The Treatment Of Psychogenic Polydipsia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch, NIMH

George Christison, M.D., Staff Fellow, Clinical Brain Disorders Branch, NIMH;
Darrell G. Kirch, M.D., Senior Staff Fellow, NPB,DIRP,NIMH;

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section On Neuropathology Studies

INSTITUTE AND LOCATION

NIMH,WAW Bldg., St Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with psychogenic polydipsia (compulsive water drinking) comprise about 6% of the schizophrenic population. These patients are usually managed by imposed fluid restriction with symptomatic treatment for episodic water intoxication. Many of these patients have been shown to have inappropriate secretion of antidiuretic hormone (SIADH) which may be a factor in their episodes of hyponatremia.

Demeclocycline is an antibiotic related to tetracycline which blocks the action of antidiuretic hormone (ADH) and is useful in treating SIADH of diverse etiologies. Preliminary study has suggested that demeclocycline might be useful in psychiatric patients with psychogenic polydipsia.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description: ZO1 MH 02393-01 CBDB

Objectives: This study is designed to evaluate the efficacy of demeclocycline in maintaining normal sodium balance in patients with psychogenic polydipsia and in preventing water intoxication.

Methods Employed: Patients are selected for participation in the study based on past history of compulsive water drinking and water intoxication. They frequently have mild chronic hyponatremia and hyposthenuria. The study incorporates a double-blind, placebo-controlled, cross-over design. The patients receive either three weeks of placebo or three weeks of demeclocycline in gradually increasing dosage. During the study, patients are examined for changes in drinking behavior, weight gain (which may indicate an abrupt increase in fluid intake) and psychosis. Serum sodium level and osmolarity are monitored. If symptomatic or severe hyponatremia develops while in the study, fluid restriction of undertaken to ensure the safety of the patient.

Major Findings: Four trials have been completed to date. The preliminary results indicate that demeclocycline is useful in modulating wide fluctuations in serum sodium level and in decreasing episodes of water intoxication which require fluid restriction.

Significance To Mental Health Research: If demeclocycline proves efficacious in decreasing episodes of symptomatic water intoxication, psychiatrists will have another method for treating this subgroup of patients. Fluid restriction requires close nursing supervision and entails a great degree of discomfort for the patient. Demeclocycline would be easier for both the staff and the patient to tolerate and may raise the level of compliance with treatment.

Proposed Course Of Project: The project will run until 10-15 trials have been completed.

Publications:

Illowsky BP, Kirch DG: Polydipsia and hyponatremia in psychiatric patients. (submitted for publication).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02394-02 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Magnetic Resonance Imaging (MRI) Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel R. Weinberger, M.D., Chief, Section on Clinical Clinical Brain studies, NPB,DIRP,NIMH

Dr. Richard Suddath, Medical Staff Fellow, Clinical Brain Disorders Branch DIRP,NIMH: Dr. John Kelsoe, Medical Staff Fellow, Clinical Neuroscience Branch, NIMH; Dr. David Pickar, Chief, Section on Clinical Studies, CNB, IRP, NIMH; Dr. Manual Casanova, CBDB, Dr. George Christison, Dr. Terry Goldberg, Dr. Fuller Torrey

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH; Section On Clinical Studies, CNB

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section On Clinical Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg., St. Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the neuroanatomical localization of the possible underlying pathology using MRI. Attempts will be made to correlated neuroanatomical abnormalities to other findings such as those seen in blood flow or BEAM studies. These studies are using state of the arts digitized image analysis system to examine gray-white matter quantitative differences between schizophrenic and normals. Preliminary results indicate that patients have larger ventricles and less temporal gray matter. This represents potentially important evidence of a relatively focal pathological process.

PHS 6040 (Rev. 1/84)

GPO 914-918

Projective Description: Z01 MH 02394-02 CBDB

Objectives: We are continuing to study the neuroanatomy of the brains of schizophrenics by using the more sophisticated non-radiologic technique, MRI, which might allow better delineation of the abnormalities that have been reported using the CT scan.

Methods Employed: In vivo MRI focuses on signals retrievable from hydrogen I. The images obtained provide better delineation of brain anatomy than the CT scan. With MRI, there is a greater gray-white matter contrast and the images in various planes are better constructed. That capacity allows the study of various brain areas that have previously been difficult to evaluate. Furthermore, as the meaning of signal intensity becomes better understood, we will be able to draw conclusions about the degree of pathology according to differences in MRI signal intensities.

Over 50 patients and normal controls have been scanned with the NIH Clinical Center machine (.5 tesla). Data have been collected and evaluated by use of a computerized system that allows area measurements on these scans. Size of ventricles, cortical areas, basal ganglia anatomy, and the anatomy of some of the forebrain nuclei are among the structures that can be evaluated using this system. The results of the first study have been submitted for publication. Total ventricular volume was significantly enlarged in the patients. In a follow-up study using a refined computerized image analysis system, we have found reduced volume of temporal gray matter.

In addition we have begun to examine discordant monozygotic twins using a more powerful 1.5 T machine that provides improved gray-white matter resolution. We have currently examined over five pairs of twins.

Significance to Mental Health Research: This study may help to determine the basic neuroanatomical lesions(s) which caused the abnormalities observed in the brains of schizophrenics, i.e., whether the pathology is related to cortical or subcortical lesions, is local or diffuse.

Proposed Course of Project: This study will continue during the next few years and it is hoped that it will be able to provide better data as to the basic pathology involved in the causation of the abnormalities seen on CT scans in the brains of schizophrenics, and to correlate there data with clinical variables.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02395-02 CBDB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Brain Imaging In Schizophrenic Patients And Normal Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Daniel R. Weinberger, .D., Chief, Clinical Brain Disorders Branch, NPB,IRP, NIMH
David G, Daniel, CBDB; Dr. George Jaskiw, Visiting Associate, Section on Clinical Brain Studies, NPB, IRP, NIH; Dr. Barbara Illowsky, Medical Staff Fellow, Section on Clinical Brain Studies, NPB, IRP, NIMH;

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section On Clinical Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg., Saint Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The project on structural brain imaging investigates structural pathology of the brains of schizophrenic patients housed in the William A. White research units using x-ray computerized tomography (CT). Patients are compared to matched normal controls. The most recent study, a culmination of four years of data collection, compared 73 schizophrenic patients to 30 normal volunteer controls. This project is a replication and extension of the previous work done in this area in the branch. Using standardized techniques four brain areas were examined: Lateral ventricles, third ventricles, cortical (parieto-occipital) areas, and prefrontal cortex. In this sample, the lateral and third ventricles continued to be significantly larger in patients than controls. A potentially exciting new finding was that though there were essentially no differences between patients and controls in cortical atrophy in the parieto occipital distribution, the schizophrenic patients showed substantially greater atrophy in the prefrontal distribution, localizing the cortical changes to this area. Further, in a subgroup of 18 drug-free and 22 medicated patients, the CT abnormalities were correlated with regional cerebral blood flow (rCBF) using the radioactive 133 Xenon inhalation technique. Relationships were found between the neurophysiological measurements and CT scanning, especially in the prefrontal cortex and ventricular areas. This work is being amplified to search for clinical and biological correlations of ventricular enlargement and prefrontal atrophy, particularly with respect to other signs of prefrontal pathology, e.g. rCBF, EEG, PET data. In addition, we followed up earlier patients, rescanning them after 9-9 years. We have found no change over time, indicating that the pathology underlying these changes is probably static.

Project Description:

Objectives: Before the advent of CT scanning, observations of the human brain were largely indirect: chemical markers of brain metabolic activity in the cerebrospinal fluid, blood, or urine; responses to centrally-acting medications; animal models of human brain function; post-mortem brain studies; and crude methods of visualizing brain structures such as pneumoencephalography. CT scanning proved a major advance in providing detailed pictures of cross-sections of brain in living subjects with minimal risk. These accurate and reliable methods could then be applied to the study of brain pathology in such diseases as schizophrenia.

We have used CT observations to study a number of parameters of brain structural abnormalities in schizophrenic patients. Reversed cerebral asymmetries and cerebellar atrophy have been described in patients, but the first and most venerable findings were those implicating atrophy of the cerebrum. In particular, previous studies have indicated enlargement of lateral and third ventricles and atrophy of the cortical surface, findings apparently unrelated to age, neuroleptic exposure or duration of illness, but apparently related to severity of illness as described by poor premorbid adjustment, diminished response to neuroleptics, poorer outcome, cognitive impairment, and other negative symptoms. Additionally, these changes are apparently present at the onset of illness.

Over the last year the laboratory has tried to meet several objectives: (i) to replicate the previous work showing cerebral atrophy in a sample of schizophrenic patients more representative of the broad distribution of affected persons. (ii) to extend the work by attempting to localize the site of cortical atrophy and (iii) to relate the changes on CT scan to physiological measures by rCBF, especially so-called "hypofrontality" (i.e., relatively diminished prefrontal blood flows under conditions of cognitive stimulation of this area).

To achieve these results, CT and rCBF data were collected along with clinical information and neuropsychological testing.

Methods Employed: Patients selected for study were housed in the clinical research units, Saint Elizabeths Hospital, William A. White Building, and were rigorously diagnosed as having schizophrenia by DSM-III criteria. Normal volunteers were obtained via several investigators at the NIH Clinical Center, Bethesda, Maryland. All subjects underwent standard CT scanning with the same GE 8800 scanner at the Clinical Center. Twelve to 13 images or slices were produced at 15° to the cantho-meatal line. Measurements were made from these images on photographic film.

Lateral ventricle size was measured using a fixed-arm planimeter, an engineering device used to measure the area of irregular two-dimensional structures. The ventricular area is divided by the area of the whole brain, multiplying by 100, giving a percentage size or ventricular-brain ratio (VBR). Third ventricular size is measured by laying a mm ruler across the greatest diameter, then multiplying by a so-called "magnification factor" of 2.7 (the relationship between the photographic image and the true size of the subject's brain). Generalized (parieto-occipital) atrophy was evaluated on an appropriate slice with a 0 (mild) to 3 (severe) scale with half-steps between (e.g., 0.5, 1.5) by referring to standard examples. Prefrontal atrophy was similarly evaluated on a scale derived from CT cuts at an appropriate

position. The patient data derived were compared to the same measurements from volunteer controls. All measurements were performed blind to patients vs controls. A subsample of patients were selected to compare CT changes and abnormalities on rCBF described in detail under another heading.

Major Past Findings: In a relatively severely-impaired sample of patients, there was evidence of abnormal enlargement of lateral and third ventricles and cortical atrophy (measured with a different scale). These abnormalities did not relate to clinical parameters such as age, duration of illness or hospitalization or neuroleptic treatment, but were correlated with poor premorbid adjustment, cognitive impairment, poor response to neuroleptics and poor outcome. Other abnormalities discovered included an increased incidence of cerebellar atrophy and reversed cerebral asymmetries in the patients. Additionally, evidence of prefrontal atrophy was correlated with increased delta-or slow - wave activity in the prefrontal area as assessed by brain electrical activity mapping (BEAM), a computerized evaluation of the electroencephalogram.

New Findings: Comparing the 73 schizophrenic patients to 30 normal volunteer controls, enlargement of lateral and third ventricles was again demonstrated; as predicted this was to a lesser degree than described before in this somewhat less-severely impaired patient sample, though still reaching statistical significance. There were no differences between patients and controls on the generalized (parieto-occpital) scale, but significant differences in the prefrontal distribution indicating a localization of atrophy in this area. This is consistent with some findings from rCBF, BEAM, Positron Emission Tomographic Scanning and Neuropsychological deficits found in schizophrenic patients. This is also significant in light of the similarities of symptoms found in person with known injuries of the dorsolateral prefrontal cortex and the so-called "core" or "defect" symptoms of schizophrenia including flattened affect, social impairment, apathy, withdrawal, etc.

Abnormalities on rCBF, in particular the so called hypofrontality seen in schizophrenic patients under conditions of specific neuropsychological stimulation (the Wisconsin Card Sort test, an activator of the dorsolateral prefrontal cortex) were compared to structural abnormalities on CT scan in 18 drug-free and 22 medicated patients. Decreased relative prefrontal blood flow was found to correlate with prefrontal atrophy and strongly with lateral ventricular enlargement all patients. This seems to indicate relationships between physiological dysfunction in the prefrontal cortex and structural abnormalities in both prefrontal and subcortical areas. This is consistent with a developing knowledge base from basic research indicating strong interrelationships between prefrontal and subcortical (periventricular) structures that may be abnormal in schizophrenia. Finally, we have rescanned 20 patients after 7-9 years and find no evidence of progression of their structural pathological condition.

Significance to Mental Health Research: Understanding the basic structural and physiological dysfunction in the brains of schizophrenic patients is vital to the progress of research in the illness. Such underpinning will allow the development of specific neurorehabilitative paradigms and potentially an understanding of the etiologies of the illness. By comparing structural and functional measurements, abnormalities can be better localized in the brain, and can be related to specific clinical parameters. The primary goal, then, is to clarify the site of the "critical lesions" in the

brains schizophrenic patients, i.e., those areas primarily effected in the illness, accounting for the core symptoms.

Proposed Course of Project: With the extensive CT data collected, correlations will be made with various clinical and psychological parameters, e.g., cognitive impairment, "negative" and "positive" symptoms and neuroleptic responsiveness. We also are utilizing sophisticated computerized image analysis system to analyze the vast amount of data contained in CT image. Plans are also being formulated to utilize an exciting new imaging technique, nuclear magnetic resonance (NMR). This technique will allow structural imaging in exquisite detail, revealing more specifically areas such as individual periventriclar nuclei, depth of cortical and periventricular gray matter, and giving a much more specific "look" at brain structural abnormalities.

Publications:

Shelton R., Weinberger D.R.: Brain morphology in schizophrenia In: Psychopharmacology: The Third Generation of Progress, Meltzer H., Bunney W., Coyle J., Davis K., Kopin I., Schuster R., Shader R. and Simpson G. (eds). Raven Press, New York, 1987, pp. 260-299.

Korpi E.R., Kaufmann C.A., Marnela K-M and Weinberger D.R.: Cerebrospinal Fluid amino acid concentrations in chronic schizophrenia. Psychiatry Research, 20:337-345, 1987.

Weinberger D.R.: Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44:660-669, 1987.

Jaskiw G.E., Andreasen N.C., Weinberger D.R.: X-ray computed tomography and magnetic resonance imaging in psychiatry. In: American Psychiatric Association Annual Review. Volume 6, Frances A.J. and Hales R. (eds). APA Press. Washington. 1987 (in press).

Weinberger D.R., Jeste D.V., Teychenne P.F. and Wyatt R.J.: Cerebral atrophy in elderly schizophrenic patients: Effects of aging and of long-term institutionalization and neuroleptic therapy. In Schizophrenia, Paranoia, and Schizophreniform Disorders of Late Life, Miller N. and Cohen G. (eds). Guilford press, 1987 (in press).

Raz S., Raz N., Weinberger D.R., Boronow J., Pickar D., Bigler E.D. and Turkheimer E.N.: Morphological brain abnormalities in schizophrenia determined by computerized tomography. A reassessment based on volumetric quantification. Psychiatry Research (in press).

Myslobodsky M.S. and Weinberger D.R.: Reversed brain anatomical asymetries in schizophrenia: A search for comtributing variables: In The Dual Brain, Levy J. et. al. (eds). MacMillan Press, London, 1986 (in press)

Shelton R.C., Doran A.R., Pickar D. and Weinberger D.R.: Cerebral structural pathology in schizophrenia. Evidence for a selective prefrontal cortical deficit American J. Psychiatry (in press).

Jaffe M.J., Bruno G., Campbell G., Lavine R., Karson, C.N. and Weinberger D.R.: Ganzfeld electroretinographic findings in Parkinsonism: Untreated

patients and the effect of L-dopa intravenous infusion. J. Neurology Neurosurgery and Psychiatry (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02397-02 CBDB

PERIOD COVERED

October 1 1986 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hierarchy and Sensitivity in Putative Frontal Lobe Tasks

PRINCIPAL INVESTIGATOR *(List other professionel personnal below the Pnncipal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Clinical Brain Disorders Branch, IRP,NIMH

Dr. Daniel weinberger, Chief Clinical brain Disorders Branch, NPB, IRP, NIMH;
Dr. John Kelso, Section on Clinical Studies, Clinical Neuroscience Branch, NIMH

COOPERATING UNITS *(if any)*

Section on Clinical Studies, Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Clinical Brain Disorders Branch

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Section On Clinical Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg., Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.33	.33	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various standardized neuropsychological tests are thought to tap frontal lobe funtion to varying degrees. These involve verbal fluency, multiple tracking, category formation, and hypothetico-deductive reasoning and set shifting. The differential performance of schizophrenic patients with physiological frontal lobe dysfunctions of these tasks is unkown. We are investigating the sensitivity of the tests and the possibility that there is a hierarchical arrangement of them.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Objectives: While there has been research regarding tasks that are sensitive to frontal lobe dysfuntion, much less is know about the relation among a bettery of putative frontal lobe tasks when administered to schizophrenic patients. We are investigating the relationship among several tasks in order to ascertain if a performance hiearchy is present and whether there is differential sensitivity of the tasks to dysfuntion. Moreover, the tasks may tap various types of frontal lobe dysfuntion.

Methods Employed: Four tests are administered to schizophrenic patients: verbal fluency, Trails B, Categories, and cut-off techniques.

Major Findings: This study is in progress.

Significance to Mental Health Reasearch: There has been renewed interest in frontal lobe symptomatplogy in schizophrenia. Tests sensitive to such dysfuntion or to various planning functions of the frontal lobe (e.g., attention, short term memory, concept formation, planning, response to feedback, deductive reasoning) may be used in elucidating both cognitive structures and quantifying the degree of dysfunction.

Proposed Course of Project: The project will continue until data collection is complete.

Publications:

Zec, R.F. and Weinberger, D.R.: Brain areas implicated in schizophrenia. In Nasrallah, H. and Weinberger, D.R. (Eds): The Neurology of Schizophrenia. Amsterdam, Elsevier, 1986, pp. 175-206.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02398-01 CBDB

PERIOD COVERED

October 1, 1987 through September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of an Auditory Sort Test

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Dr. Terry Goldberg, Special Expert, Clinical Brain Disorders Branch, IRP,NIMH

Dr. Daniel weinberger, Chief Clinical Brain Disorders Branch, Chief, Clinical brain Disorders Branch, NPB, IRP, NIMH; Dr. Craig Karson, Staff Staff Psychiatrist, NPB, IRP, NIMH; Dr. Karen F. Berman, Staff Psychiatrist, NPB, IRP, NIMH

COOPERATING UNITS *(if any)*

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Clinical Brain Disorders Branch

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NIMH, Saint Elizabeths Hospital, Washington, D.C.

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
.33	.33	0

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Concepts formation tasks with a set shifting component have proven sensitive to frontal lobe dysfunction. The Wisconsin Card Sort, perhaps the most widely known test of this class, activates prefontal regions in most normal but no schizophrenic subjects. It is presented in the visual modality. To futher validate the use of such a test and to facilitate cognitive activation in EEG-BEAM studies while reducing eye movement, an auditory analog of the card sort was developed.

GPO 914-918

Project Description:

Objectives: The Auditory Sort Test is a non-verbal auditory task. It has two funtions. The first is to differentially activate brain regions, namely temporal and prefrontal. The second is to display adequate psychometric properties(reliability, concurrent validty with Wisconsin Card Sort, independence from motivation and attentional factors, and separation of groups).

Methods Employed: Tones are presented to the subject and she/he is asked to match each to one of two target tones. Tones may be matched on the basis of volumem pitch, or duration. After the subject makes 10 successive correct matches, the categorization shifts without. There are 60 items in the test and it askes about 10 minutes to administer. Normal and schizophrenic subjects are administered the test along with the Wisconsin Card Sort and the Continuous Performance Task (of vigilance and attention).

Major Findings: This study is in progress.

Significance to Mental Health Research: The Auditory Sort Test may prove a significant tool in activating specfic regions of the brain. Moreover, performance among different groups (schizophrenic, neurologic, normal) may be a sensitive indicator of dysfunction.

Proposed Course of Project: The project will continue until a data collection is complete. Patients undergoing both cerebal blood flow and computerzied EEG will receive the test (in a time locked format for the latter). Further studies on neurologic patients with discrete regions and non-schizophrenic psychiatric are planned.

Publications:

Weinberger, D.R.: The pathogenesis of schizophrenia: A neurodevelopmental theory. In Nasrallah, H. and Weinberger, D.R. (Eds.): The Neurology of Schizophrenia Amsterdam, Elsevier, 1986, pp. 397-406.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02399-02 CBDB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Postmortem Brain Tissue Examination in Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joel E. Kleinman, M.D., Ph.D., Deputy Chief, CBDB, IRP, NIMH

Emanuel Casanova, M.D., Neuropathologist, CBDB; Daniel R. Weinberger M.D.,Chief, CBDB, Craig N. Karson, M.D., Staff Psychiatrist, CBDB, George Jaskiw, M.D., Staff Psychiatrist, Natanel Zelnik, M.D., Guest Worker, CBDB, Steven Paul,M.D., Chief, Clinical Neuroscience Branch, Farouk Karoum, Ph.D., Chemist, NPB, IRP, NIMH and Markku Linnoila, M.D.,Ph.D., Chief, NIAAA, NIMH;

COOPERATING UNITS *(if any)*

NIAAA; Neuroscience Branch, NIMH; Neuropsychiatry Branch

LAB/BRANCH

Clinical Brain Disorders Branch

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Section On Neuropathology Studies

INSTITUTE AND LOCATION

NIMH, WAW Bldg., St Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Postmortem Studies in neuropsychiatric disorders test hypotheses with regard to schizophrenia, suicide, Parkinson's Disease, addictions and aging. New findings include the following: Decreased numbers of D-1 dopamine receptors with increased affinity in schizophrenic patients in the caudate nucleus as compared with controls; (2) No evidence for transmission of viruses from schizophrenic brain specimens to controls; (3) Little evidence for lateralization of neuropeptides (somatostatin, and vasopressin, oxytocin) in schizophrenic brain samples and; (4) decreased dopamine reuptake binding in Parkinson's patients versus controls.

GPO 914-918

Objectives: Neurochemical analyses of postmortem human brain tissue is an area of increasing interest to psychiatric research. Many groups are now concentrating on mapping central neuronal pathways or identifying biochemical abnormalities in neurological and psychiatric diseases. Our studies have focused primarily on neurochemical hypotheses in schizophrenia and suicide with some work on aging, Parkinson's disease and addiction. In the schizophrenic syndrome our efforts have focused mostly on catecholamines, (especially receptors), indoleamines, neuropeptides and amino acids. Viral hypotheses have also been tested using schizophrenic brain specimens. In suicide studies, the emphasis has been on indoleamines, norepinephrine, acetylcholine, amino acids, and proteins. In aging, studies have examined dopamine receptors (types 1 and 2) and dopamine reuptake mechanisms. The latter has been the focus of Parkinson's disease studies. Lastly, addiction studies have focused on PCP and opiate binding sites.

Methods Employed: Brains are collected seven days a week from the D.C. Medical Examiners office by the Section Chief, the neuropathologist and assistants. Patient and control brains are dissected by the medical staff fellow and a research assistant according to international criteria. Careful matching for postmortem interval and for freezer storage time is done in addition to the routine age, race, and gender matching. Another variable that may require matching is the time of year at death. Diagnosis of patients is performed independently by two psychiatists, using the newly developed Diagnostic Evaluation After Death criteria.

New Findings: Schizophrenia studies: (1) Decreased dopamine type 1 receptors with increased affinity in the caudate nucleus of schizophrenic patients; (3) No asymmetries of vasopression, somatoslatin oxytocin in a number of brain regions; (3) No abnormalities in amino acids; (4) No evidence for viral transmission.

Suicide Studies; (1) Preliminary evidence for abnormal phosphorylation of proteins in parietal cortex of non-schizophrenic suicides; (2) Normal amino acids in non-schizophrenic suicide brains.

Aging Studies: Decreases with age in dopamine type 1 and type 2 receptors and presynaptic dopamine reuptake binding sites.

Parkinson's Disease Studies: Decreased presymaptic dopamine reuptake sites in caudate nucleus consistent with loss of nigral innervation.

Addiction Studies: (1) Preliminary evidence for phosphorylation protein abnormalities in alcoholics; (2) Normal PCP and opiate binding sites in brains of heroin addicts.

Significance to mental Health Research: These studies are designed to elucidate neurochemical and structural abnormalities in the brains of schizophrenics, suicides, alcoholics, heroin addicts and patients with Parkinson's disease. This understanding will hopefully lead to new treatment and prevention.

Proposed Course of Project: Schizophrenia (1) Attempts to replicate and examine dopamine receptors in other brain regions are currently underway; (2) another project in progress involves measurements of catecholamines in a number of limbic nuclei; (3) attempts to measure glutamate receptors are

being planned; (4) new methods to study brains with neuronal morphometrics, immuno-cytochemistry, autoradiography and in situ DNA hybridization are in progress or are planned.

Suicides and Alcoholism: Studies looking at phosphorylated proteins are in progress. Further studies looking at indoleamines and metabolites are planned for better diagnosed patients.

Heroin Addiction: Further work on opiate receptors and endogenous opiate compounds are planned.

Publications:

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Weinberger, D.R. and Kleinman, J.E.: Chapter 3: Observations on the brain in schizophrenia. In: Frances, A.J. and Hales, R.E. (eds): Psychiatry, Updates, The American Psychiatric Association Annual Review, Vol. 5. Washington, D.C., American Psychiatric Press, Inc., 1986,pp. 42-67.

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Zelnik, N., Angel, I., Paul, S.M. and Kleinman, J.E.: Decreased density of human striatal dopamine uptake sites with age. European Journal of Pharmacology 126:175-176, 1986.

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Bracha, H.S., Shultz, C., Glick, S.D. and Kleinman, J.E.: Spotaneous asymmetric circling behavior in hemi-parkinsonism: A human equivalent of the lesioned-circling rodent behavior. Life Sci. 40: 1127-1130, 1987.

Hess, E.J., Bracha, H., Kleinman, J,E, and Creese, I. Dopamine receptor subtype imbalance in schizophrenia. Life Sci. 40: 1487-1497, 1987.

Lake, C.R., Kleinman, J., Kafka, M.S., Ko, G., Moore, S. and Ziegler, M. Chapter 8. Norepinephrine metabolism in schizophrenia. In: Nasrallah, H. and Delisi, L.E. (eds.): Handbook of Schizophrenia, Volume II: Neurochemistry and Neuropharmacology. Amsterdam, The Netherlands, Elsevier Science Publishers, 1987, pp. 1-30.

Kleinman, J.E., Jaeger, A.-C., Bracha, H.S. and Fernstrom, J.D.: Left-right comparisons of neuropeptides in human brain: Preliminary results. In: Flor-Henry, P. and Gruzelier, J. (eds.): Cerebral Dynamics, Laterality and Psychopathology. Developments in psychiatry-volume 8. Amsterdam, The Netherlands, Elsevier Science Publisher, 1987, pp.

Korpi, E.R., Kleinman, J.E., Goodman, S.I. and Wyatt, R.J.: Neurotransmitter amino acids in postmortem brains of chronic schizophrenic patients. Psychiatry Res. (in press).

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Kaufmann, C.A., Weinberger, D.R., Stevens, J.R., Asher, D.M., Kleinman, J.E., Sulima, M.R., Gibbs, C.J. and Gajdusek, D.C.: Intracerebral inoculation of experimental animals with brain tissue from schizophrenic patients. Failure to observe behavioral and neuropathological effects. Arch. of Gen. Psychiatry (in press).

Rothman, R.B., Bykov, V., Cadet, J.L. and Kleinman, J.E.,: A postmortem study of the effect of chronic opiate abuse on psychotomimietic binding sites of human frontal cortex. Neuropeptides (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02400-01 CBDB

PERIOD COVERED
October 1, 1986 throught September 30, 1987

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*
Eight Year Follow-up Of Ventricular Size In Schizophrenia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Pnncipal Investigator.) (Neme, title, leboretory, and institute effilietion)*

Barbara P. Illowsky, M.D., Medical Staff Fellow, Clinical Brain Disorders Branch, NPB, IRP, NIMH

Denise M. Juliano, MSW, Social Worker, Clinical Services Branch, WAW Division; Dr. Llewellyn B. Bigelow, Associate Clinical Director, WAW Division, Saint Elizabeths Hospital, NIMH; Daniel R. Weinberger, M.D., Chief, Clinical Brain Disorders Branch, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section On Neuropathology Studies

INSTITUTE AND LOCATION
NIMH, WAW Bldg., St. Elizabeths Hospital

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	3	2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cranial computerized axial tomography (CT) has been useful in identifying a subgroup of schizophrenic patients with enlargement of the lateral ventricles and frontal cortical atrophy. The relationship between these anatomic abnormalities and mental illness remains speculative, but it has been suggested that such enlargement may be more common in those patients with more severe illness or poor prognosis. Previous CT studies have shown no correlation between ventricular size and duration of illness or age and a single prospective study failed to show progressive ventricular enlargement over a brief, 3 year, follow-up period.

We rescanned a cohort of schizophrenic patients 7-9 years after a pilot study of ventricular size. This study greatly extends the follow-up period. No pattern of change in ventricular size or frontal cortical atrophy was seen, indicating that the pathology underlying these changes appears to remain static.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives: This project was designed to determine if schizophrenic patients have progressive cerebral atrophy or ventricular enlargement over an 8 year period, and to determine if ventricular size, or changes in ventricular size correlated with their clinical status.

Methods Employed: Locally residing patients who had participated in a 1979 study of CT scanning in schizophrenia were contacted. 18 patients consented. CT scans were obtained on an EMI 1010 scanner located at St. Elizabeths Hospital. This is the same model scanner used in the original study. Lateral ventriculr size was determined by mechanical planimetry. Frontal atrophy was assessed by a visual scale. Patient charts were reviewed for clincial information.

Major Findings: No consistent patterns of change were seen in ventricular size or configuration, or in cortical atrophy. Ventricular size did not correlate with the age of the patient, duration of hospitalization or length of illness.

Significance To Mental Health Research: This study provides direct information on an anatomic abnormality present in a subgroup of schizophrenics. It indicates that the pathology associated with lateral ventricular enlargement and cortical atrophy is not progressive. It suggests therefore, that cerebral structural abnormality probably occurs early in the patients' course, and that schizophrenia is not a progressive degenerative disorder.

Proposed Course Of Project: The project is completed.

Publications:

Illowsky BP, Juliano DM, Bigelow LB, Weinberger DR: Stability of CT scan findings in schziophrenia: results of an eight year follow-up study. Journal of Neurology, Neurosurgery, and Pyschiatry (in press).

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